

The Combined Use of Radiotherapy and Capsaicin in the Treatment of Colorectal Cancer Cells Line

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ABSTRACT

Colorectal cancer is the second most frequent kind of cancer worldwide. The treatment may comprise surgery, chemotherapy, and radiation therapy. More than half of cancer treatments are derived from natural sources, such as capsaicin. The biggest challenge is to get the disease under control as much as possible while causing as little harm to the healthy tissues around it. Cells were treated with an extract, Capsaicin of (chili peppers) and irradiated at 1 Gy, 3 Gy and 5 Gy. The MTT test was used to determine viability, and absorbance was measured at 570 nm using a Counter reader (Mutiskan EX, Thermo Lab systems). Capsaicin extract has shown a potential radiosensitizing effect in vitro for colorectal cancer. The inhibition effect in the cell lines was not selective and is significantly different from conventional radiotherapy. Capsaicin extract from chili peppers concerted with radiation proved more effective in colorectal cancer cells than radiotherapy alone.

Keywords – Colorectal cancer, CaCo-2, HDFn, Capsaicin extract, radiotherapy.

1. INTRODUCTION

Cancer is the main cause of mortality in advanced nations in 2020, surpassing heart disease [1]. Human colorectal cancer is the fourth most often diagnosed kind of cancer globally. It manifests as a malignant tumor in the colon or rectum mucosa [2]. CRC is also one of the main causes of mortality, and although our understanding of the illness has improved in recent years, existing therapies are insufficient to control metastatic types of CRC [3]. Surgery is the primary option for individuals with possibly curable CRC, however depending on the stage of the illness, neoadjuvant chemotherapy and/or radiation may be administered before or after surgery. However, these treatment regimens are insufficient to control CRC since 30% of patients with stage I-III and up to 65% of patients with stage IV will acquire recurrent illness, emphasizing the need of developing new and more effective treatment approaches [4].

Radiotherapy has long been the conventional treatment technique for several malignancies, including colorectal cancer. Although an acceptable response to radiation therapy is obtained during the first phase of cancer treatment, many cancer patients exhibit radiation resistance [5]. Today, radiation therapy ratings can be based on how well radiosensitizers improve the effectiveness of treatment.

The potential of nutraceutical natural chemicals such as flavonoids, anthocyanidins, carotenoids, and terpenoids for cancer prevention has been extensively studied, and there is enough evidence to suggest that moderate fruit and vegetable diet is associated with a lower risk of CRC [6]. Some members of these families

of chemicals have the capacity to control signaling pathways and to affect the expression of genes involved in cell [7]. In addition to their potential for prevention, certain of these molecules may also be beneficial for the treatment of CRC, particularly when used in conjunction with other medications.

Combination treatment allows for the simultaneous targeting of several cancer pathways, leveraging various mechanisms of action to diminish tumor drug resistance [8]. The DNA mismatch repair phenotype (around 15% of CRC cases), chromosomal instability versions (CIN; around two-thirds of cases), and other less common CRC versions like abnormal DNA methylation, colon inflammation status, and microRNA triggering effects all contribute to CRC development [9]. Several studies published in recent years have indicated that cancer therapy by combinatorial strategy is far more successful than the usage of medications separately [10]. Furthermore, chemosensitization by means of phytochemicals, based on the use of a natural substance to boost the effectiveness of a medication by modification of its resistance pathways, is one of the treatments recommended to overcome chemoresistance, one of the primary obstacles in CRC therapy [11]. The aim of this research was to assess the cytotoxic effect of a plant extract (Capsaicin) combined with radiation in colorectal cancer cell lines.

2. MATERIAL AND METHOD

2.1 Capsaicin extraction from hot pepper

Several organic solvents may be used to extract capsaicin from spicy peppers, but only ethanol is adequate to produce pharmaceutical-grade material [12,13]. The capsaicin was extracted from dried and crushed *Capsicum annum*, which was stored in desiccators [14]. Extraction was carried out in a water bath at 40°C for 5 hours using 96% (v/v) ethanol and 0.1-0.5 g of powdered plant material. Then, water vacuum filtration for obtaining an ethanol extract of capsaicin [15].

2.2 Cell lines and Cell Cultures

Human colorectal cancer cells (CaCo-2) and normal human dermal fibroblasts (HDFn) were bought from Pasteur in Iran. The cells were cultured in RPMI 1640 culture media supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37°C, 5% CO₂, and 100% humidity. The cells were kept in an exponential growth phase by changing the media every 2–3 days. When the cells had achieved around 80% confluence, they were trypsinized, collected, and seeded into a new tissue culture flask. Cell survival rates were measured using the MTT test. For this reason, cells were sown in 96-well plates and left to adhere for 24 hours before being exposed to the proposed studies.

2.3 Irradiation Setup

After 24 hours of incubation at 37°C, giving the cells enough time to fully attach to the surface, the cells were treated with Plant extract (Capsaicin) and radiation in linear accelerator (Elekta Synergy 3630, LINAC, Imam Hassan Al Mujtaba Hospital, Iraq). Cells were treated with 6 MeV photons at a dosage rate of 3 Gy/min. The cells were subjected to total doses of 1, 3, and 5 Gy with a 10×10 cm² field size. 3 cm thickness of a Perspex sheet was placed on top of the six wells plate and three centimetres of a Perspex sheet was utilized under the bottom of plate as a source of backscatters and Source surface distance (SSD) 100 cm photons. After irradiation, the cells were cultured for two days at 37°C in a humidified 5% CO₂ environment. The cells were then frozen and stained with 0.4% crystal violet, and visible colonies containing more than 50 cells were counted. The inhibition rate was computed based on the survival of the non-treated group and the survival percentage of the treatment group by following formula: $\text{Inhibition rate} = [(O.D. (\text{control}) - O.D. (\text{sample})) / O.D. (\text{control})] \times 100\%$.

2.4 MTT Assay

The MTT test was measured as previously reported (Mosmann, 1983). After irradiation, cells were treated with MTT reagent for one to two days. The Mutiskan EX microplate reader (Thermo Lab systems) was employed to measure the absorbance at 570 nm. The viability of control cells (untreated cells) was assumed to be 100%.

2.5 Statistical Analysis

The statistical analysis was carried out using GraphPad Prism version 9.2 (GraphPad Software Inc., La Jolla, CA). A one-way ANOVA (Tukey's Test) was employed to establish whether or not group variance was significant. The Shapiro-Wilk test was used to establish the normal distribution of quantitative parametric data, which was reported as mean \pm SD. Significant differences were defined as * $p < 0.05$ and ** $p < 0.01$.

3. RESULTS

3.1 Effect of Radiotherapy and Capsaicin on CaCo-2 and HDFn Cells Proliferation for 24 h

To assess the effect of radiotherapy and Capsaicin on cell viability in CaCo-2 colorectal cancer cells, we used MTT assay. Cell viability and proliferation were evaluated at 24 h after exposure at 1, 3, and 5 Gy and Capsaicin at a concentration of 100 $\mu\text{g/ml}$ using one assays. The viability of CaCo-2 cells was significantly ($p < 0.05$) reduced following radiotherapy irradiation and add Capsaicin. As shown in table 1, the reduction in cell viability between the three doses, extract of capsaicin and Combining radiotherapy and plant extract was different 24 h after treatment. Notably, 24 h after irradiation and treatment by Capsaicin, cell viability gradually decreased in a dose-dependent manner by 93.27 %, 86.01, and 79.84 % in the 1, 3, and 5 Gy and 51.06% with Capsaicin at concentration 100 $\mu\text{g/ml}$ groups compared to untreated cells, respectively. while, the MTT assay revealed combination radiation with and Capsaicin affected the viability of CaCo-2 cells 24 h after exposure to three doses with Capsaicin. In addition, as shown in Table 1, and Figure 1 the growth rate of radiotherapy-exposed cells decreased compared to that of non-irradiated cells.

In HDFn normal cells, the results of the MTT test after 24 hours showed that the use of radiation inhibited cells by 5.27%, 7.31%, and 8.76% at doses (1, 3 and 5 Gy), respectively, while Capsaicin inhibited normal cells with an inhibition rate of 25.71 %, while the decrease in the percentage of vitality of cells exposed to the three doses of radiotherapy was with Capsaicin 35.17%, 40.34% and 48.75% in doses, respectively. as shown in table 1. Exposing normal cells HDFn to radiotherapy showed a significant effect (p value < 0.0001), and Capsaicin alone showed a significant toxic effect (p value < 0.0001), with a greater decrease observed in colorectal cancer cells line when combination with capsaicin at a concentration of 100 $\mu\text{g/ml}$, these findings are shown in Figure 2.

Table 1: Effect of different doses of radiotherapy and Capsaicin the HDFn viability and cells of CaCo-2 using the MTT assay for 24 hours exposure at a temperature of 37 °C.

	Cell Viability % \pm SD		Sig.	p Value
	HdFn	Caco-2		
"Untreated Cells"	96.55 \pm 1.06 a	94.73 \pm 0.94 a	ns	0.96
"1 Gy"	94.73 \pm 0.72 a	93.27 \pm 0.77 a	ns	0.99
"3 Gy"	92.69 \pm 1.33 a	86.01 \pm 2.39 b	**	0.01
"5 Gy"	91.24 \pm 2.09 a	79.84 \pm 3.03 c	**	<0.0001
Capsaicin	74.29 \pm 2.78 b	51.06 \pm 2.49 d	**	<0.0001
"1 Gy + Capsaicin"	64.83 \pm 5.26 c	59.24 \pm 3.69 d	*	0.03
"3 Gy + Capsaicin"	59.66 \pm 6.16 c	49.63 \pm 3.82 e	**	<0.0001
"5 Gy + Capsaicin"	51.25 \pm 5.22 d	41.78 \pm 3.59 f	**	<0.0001

Different letters (a, b, c, d, e, f) in row are significant at $p \leq 0.05$. **: $p \leq 0.01$, * $p \leq 0.05$, NS: Non-Significant, SD: Standard Deviation.

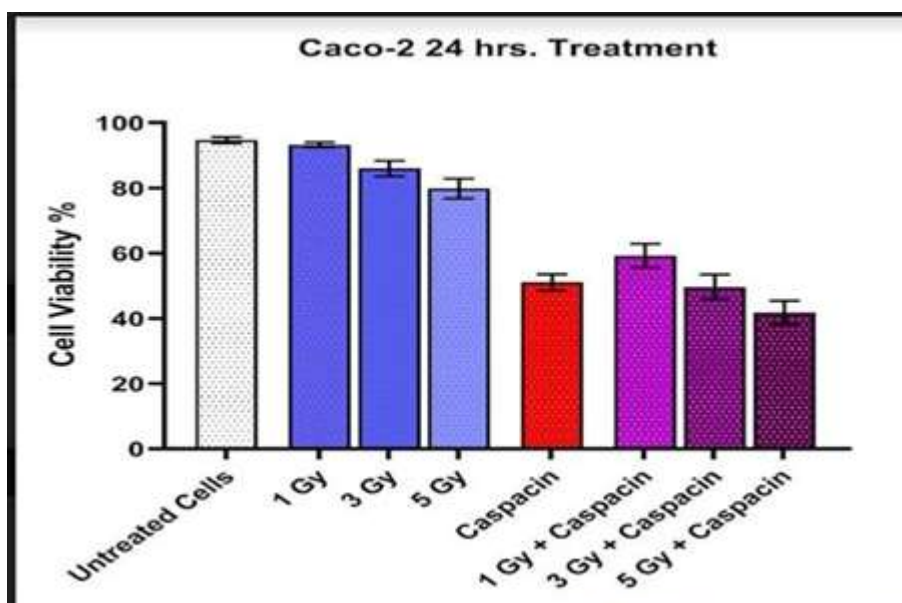


Fig 1: The different doses Effect of radiotherapy and Capsaicin on the CaCo-2 cells viability during using MTT assay for 24 hours exposure period at a temperature of 37 °C.

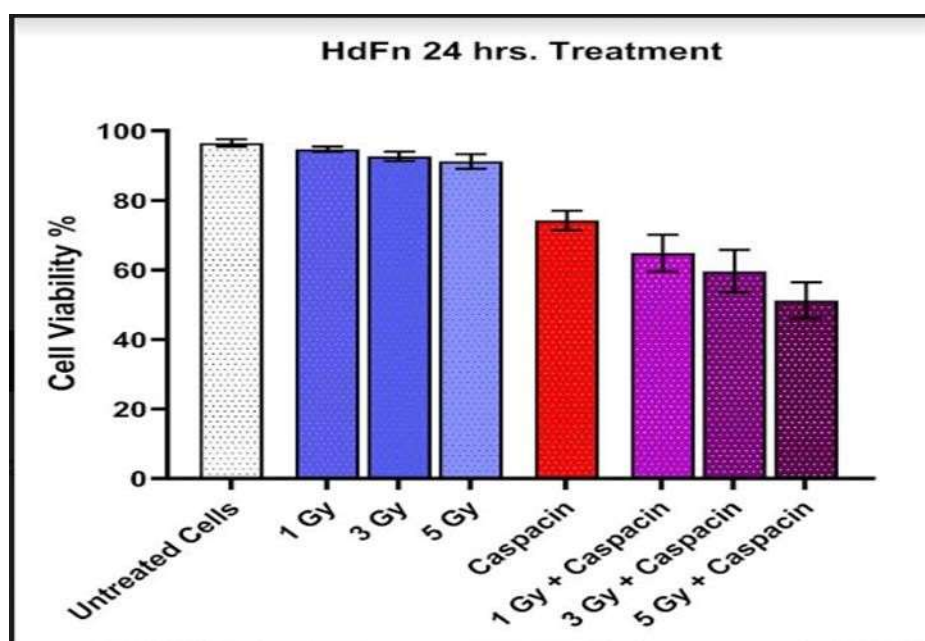


Fig 2: The different doses Effect of radiotherapy and Capsaicin on the HDFn cells viability during using MTT assay for 24 hours exposure period at a temperature of 37 °C.

3.2 Induction of apoptosis by radiotherapy and Capsaicin alone and in combination in CaCo-2 colorectal cancer cells and HDFn normal cells for 48 h

The MTT assay was used to examine the effects of CAP and radiotherapy on the viability of two cell lines (CaCo-2 colorectal cancer and HDFn normal cells) in vitro. one concentrations of CAP and three doses of radiotherapy were used to treat cells lines for 48 h. As shown in table 2. Both CAP treatment alone and radiotherapy treatment alone caused a dose and concentration-dependent decrease in the viability of the cell lines. After 48 hours of treatment, the rate of CAP inhibition was 28.49% in HDFn cell lines and 52.18% in CaCo-2 colorectal cancer. In addition, the values inhibition of radiotherapy (1, 3 and 5 Gy) in CaCo-2 and

HDFn cell lines were 11.86%, 15.8% and 21.3% in CaCo-2 and 7.16%, 9.2% and 9.5% in, respectively. a MTT assay was used to evaluate whether a concentration of CAP can enhance the radiotherapy-induced toxicity in CaCo-2 and HDFn cells. As our previous results indicated that relatively low doses of radiotherapy exhibited mild cytotoxicity and could not induce significant apoptosis in Colorectal cancer cells and normal cells, we performed viability tests of CaCo-2 and HDFn cells treated with concentration of CAP (100 µg/ml) in combination with radiotherapy (1, 3 and 5 Gy) for 48 h to explore the effects of the combination. As shown in Fig. 1c-e, compared to radiation alone, the combination of radiation and CAP induced significantly higher cytotoxicity in the cell lines. As shown in Figs. 3, 4 and table 2.

Table 2: Effect of different doses of radiotherapy and Capsaicin the HDFn viability and cells of CaCo-2 using the MTT assay for 48 hours exposure at a temperature of 37 °C.

	Cell Viability % ± SD		Sig.	p Value
	HdFn	Caco-2		
"Untreated Cells"	93.92 ± 1.52 a	92.63 ± 1.64 a	ns	0.9965
"1 Gy"	92.84 ± 1.27 a	88.14 ± 1.79 ab	ns	0.1324
"3 Gy"	90.8 ± 1.4 a	84.2 ± 3.1 b	ns	0.0694
"5 Gy"	90.5 ± 2.1 a	78.7 ± 3.9 c	**	<0.0001
Capsaicin	71.51 ± 2.49 b	47.18 ± 2.15 d	**	<0.0001
"1 Gy + Capsaicin"	62.5 ± 5.9 bc	56.1 ± 4.9 e	*	0.0231
"3 Gy + Capsaicin"	57.8 ± 7.72 c	47.9 ± 2.8 d	**	<0.0001
"5 Gy + Capsaicin"	48.9 ± 4.4 d	40.9 ± 3.51 f	**	0.0022

Different letters (a, b, c, d, e, f) in raw are significant at $p \leq 0.05$. **: $p \leq 0.01$, * $p \leq 0.05$, NS: Non-Significant, SD: Standard Deviation.

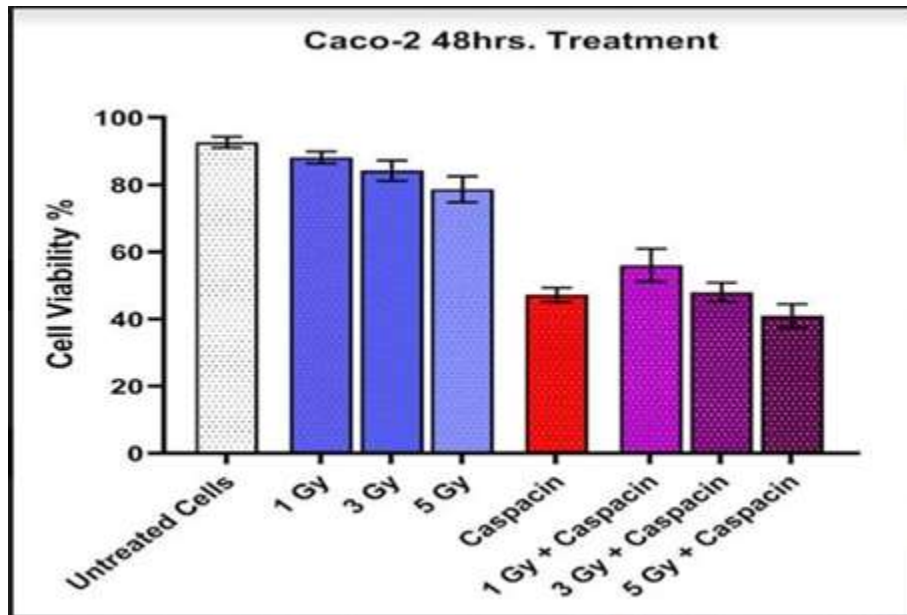


Fig 3: The different doses Effect of radiotherapy and Capsaicin on the CaCo-2 cells viability during using MTT assay for 48 hours exposure period at a temperature of 37 °C.

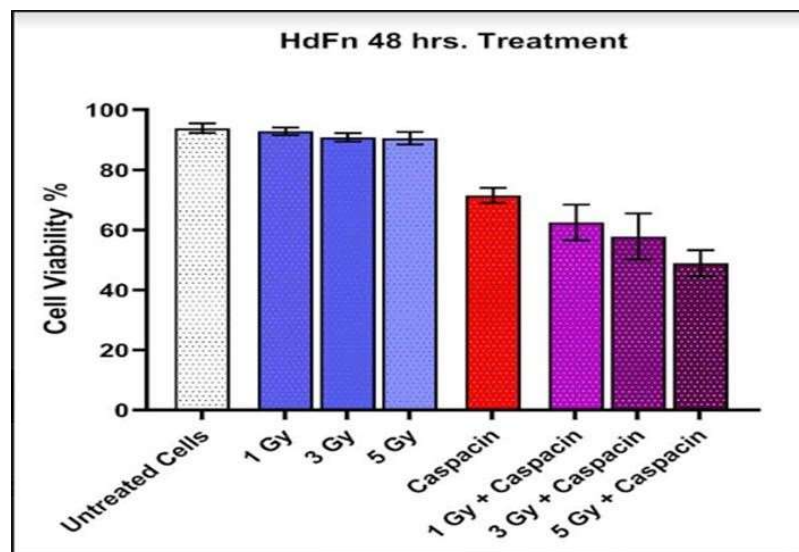


Fig 4: The different doses Effect of radiotherapy and Capsaicin on the CaCo-2 cells viability during using MTT assay for 48 hours exposure period at a temperature of 37 °C.

4. Discussion

In the context of the preceding information and drawing on previously collected data on the various cell lines, this study presents an integrated investigation of the response of Caco-2 andHdFn cells to X-ray doses up to 5 Gy. Radiation-induced cell viability is a crucial metric in biological research. MTT test is a simple and effective technique for evaluating cell growth and viability. According to statistics, more than 50% of cancer patients need radiation treatment, with pelvic and abdominal malignancies accounting for more than half [16]. Radiation therapy, one of the major therapeutic approaches for cancer treatment, may be considered a "double-edged sword" in that it may lead to genetic alterations in exposed normal tissue, but it may also cause tumor cell death by destroying the DNA of exposed tumor tissue [17]. That is, even if radiation treatment targets tumour cells as anticipated, it may unavoidably kill healthy cells [18]. Many research have demonstrated that abdominal radiation treatment may cause radioactive intestine illnesses, which present mostly as acute and chronic intestinal injuries [19].

In the present research, 24 hours after radiation exposure, cell viability decreased in a dose- dependent manner compared to non-irradiated cells. However, the MTT assay showed that cell proliferation was significantly reduced only 24 hours after radiation exposure compared to non-irradiated cells. These findings might be due to the properties of cell growth tests [20]. Communicated that radiotherapy (1, 3, and 5 Gy) stimulates the break or eliminate of continuously strained lines of occludin in cells lines [21]. Caco-2 viability was assessed using the MTT test and found to be as high as in the sham condition for cells irradiated with varying dosages of radiation and followed for up to 48 hours after exposure. The proportion of dead cells was discovered to increase in a dose-dependent way. Findings at 5 Gy are highly consistent with what was seen in a prior study following X-ray exposure of Caco-2 cells seeded 48 h before irradiation [22].

In a prior study, we discovered that capsaicin administration dramatically increased growth arrest and apoptosis in human colorectal cancer cells [23]. However, the fundamental processes by which capsaicin impacts human colorectal cancer are still poorly understood. This study's concentration (100 μ M) corresponds with prior studies on human colorectal cancer cells in vitro [24,25]. The combination of CAP with chemotherapeutic medications or radiation treatment may give techniques for increasing chemotherapy or radiation therapy sensitivity, lowering harmful side effects, and overcoming chemotherapy resistance and radiotherapy tolerance. In light of the aforesaid results, it may be hypothesized that CAP may become an auxiliary technique to established treatment approaches like as chemotherapy or radiation in the near future [26].

Capsaicin has been shown to have anti-cancer properties, including anti-proliferation, activation of apoptosis and autophagy, anti-angiogenesis, and anti-metastasis. More studies is required to determine the safety and effectiveness of capsaicin, as well as its potential anti- tumor effects when coupled with other conventional medicines or radiation [26].

5. CONCLUSION

Active natural substances have anticancer effects in vitro and in vivo via many mechanisms and routes. Organic compounds mixed with conventional therapy may increase sensitivity, reduce medication dose, minimize treatment resistance, and mitigate the side effects of conventional therapies. It has the potential to be an important therapeutic tool for treating colorectal cancer development and metastasis.

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