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Combined radiotherapy, plant extracts, and chemotherapy in colorectal cancer cells

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ABSTRACT

Colorectal Cancer (CRC) is a kind of cancer that is the second most common in the world, and its treatment is surgery, chemotherapy and radiation therapy. Therefore, this study investigates Fraxetin and Capsaicin's ability to prohibit CRC cells from reproducing through radiation therapy, and thus, it can serve as a viable substitute for cisplatin in CRC treatment. The largest defy is to increase the destruction of the tumour while decreasing the destruction of healthy tissue and the side effects on the surrounding normal tissues. Cells were cured with Capsaicin and Fraxetin extracts from chilli peppers and Fraxinus and radiated at 1 Gy, 3 Gy and 5 Gy. Viability has been assessed by MTT test and quantified at 570 nm in a Mutiskan EX (Thermo Lab systems). Cisplatin, conventional chemotherapy, was utilised as a positive control. Utilise of Fraxetin and Capsaicin extract showed a prospective radio-sensitising impact in a laboratory for CRC. The inhibition impact in the cell line wasn't eclectic and is extremely analogous to the impact of traditional chemotherapy. The capsaicin of chilli peppers, incorporated with radiation therapy, has been the most active extract for CRC cells.

Keywords: Colorectal Cancer (CRC), Calcium Carbonate (Caco-2), radiotherapy, chemotherapy, Human Dermal Fibroblasts (HDFn), fraxetin, capsaicin

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INTRODUCTION

Cancer is a widespread illness around the globe whereby specific cells of the body proliferate out of command, divide faster than normal cells, are unorganised, and may spread to all body regions. Cancer cells may attack any place in the body, which is made up of a massive number of cells, reaching trillions of cells [1]. Human cells grow and divide through an action known as cell division to generate new cells as needed by the human body.

Colorectal Cancer (CRC) is one of the more frequent kinds of cancer in Western societies, and it is the world's second leading cause of cancer related death [2, 3]. The International Agency for Research on Cancer (IARC) estimates that the number of new cases of CRC will increase by 63%, to 3.2 million per year by 2040, while the mortality rate will increase by 73%, to 1.6 million per year [4]. The worldwide risk of CRC increases primarily due to a senility population and the greater adoption of westernised diets in developed and developing nations [5, 6].

Surgery, chemotherapy, and radiation therapy are the primary therapeutic techniques used in clinical practice to treat CRC [7-9]. Radiation therapy has the power to shrink and kill cancer cells by bombarding them with ionised radiation and causing Deoxyribonucleic Acid (DNA) damage either directly or indirectly via the creation of Reactive Oxygen Species (ROS). It is critical to raise the radiation dosage to cause significant damage to targeted cancer cells [10, 11]. Since ionising radiations cannot discriminate between normal and malignant cells, radiations may cause biological harm in natural tissues. As a result, the dosages should be bounded beneath the therapeutic level to protect the natural surrounding tissue [12-14]. As a result, the repetition of CRC is observed in more than 50% of status [15]. Amongst them, systemic chemotherapy is esteemed as a promising therapeutic process, according to its capacity to trigger a good therapeutic response, ameliorate quality of life and protract survival [16, 17].

Cisplatin is one of the most considerably utilised chemotherapies, whose extends a sturdy therapy impact, but some tumour kinds, such as colon, ovarian and lung cancers, have not shown satisfactory outcomes in response to cisplatin [18]. As a result, improving the effectiveness of certain compounds may contribute to the treatment of cancer using Cis Diamminedichloroplatinum (CDDP) chemotherapy. The research has focused on developing combination applications of CDDP with other safe and efficacious agents.

Herbal medications or normal substances are helpful in the treatment of several forms of cancer, whether administered as a monotherapy or in combination with radiation and conventional chemotherapy [19]. As a result, finding medications to cure cancer is becoming more vital. Capsaicin has recently been shown to destroy different tumour cells effectively. Furthermore, capsaicin also reduced the proliferation of human leukemic cells, gastric, nasopharyngeal prostate, and hepatic carcinoma cells in vitro due to its capacity to trigger cell cycle arrest and induce cell death [20-22]. Fraxetin is a plant-derived coumarin isolated mostly from Fraxinus bungeana that has been shown to have powerful anti-inflammatory, antibacterial, and neuroprotective properties with little toxicity [23, 24]. Increasing data supports fraxetin's pharmacological activity, particularly its neuroprotective properties in type 2 diabetes and anti-inflammatory effects caused via lipid peroxidation inhibition [25, 26].

Multiple studies have shown that fraxetin destruction non-small-cell lung cancer, breast cancer, and osteosarcoma by G1-phase cell cycle arrest, apoptosis induction, and destruction of M2 macrophage differentiation [27]. However, few research has examined fraxetin's impact on gastrointestinal cancer, including its targets and mechanism of action. This research aimed to assess the inhibition activity of plant extracts (Fraxetin and Capsaicin) related to radiation therapy in human cell lines of colorectal and the possibility of using Fraxetin and Capsaicin with radiation therapy instead of cisplatin for CRC treatment.

MATERIAL AND METHOD

Cell culture and viability assessment

Human Colorectal Cancer Cells, Caco-2 and natural Human Dermal Fibroblasts (HDFn) were acquired from Pasteur in Iran. Cells were cultivated in RPMI-1640 culture media with 10% fetal bovine serum and penicillin/streptomycin at 37°C, 5% CO_2, and saturation moisture. The cells were kept in an exponential development stage by mutating the media each 2 days-3 days. The cells were trypsinised, gathered, and seeded into a tissue culture flask when they attained 80% concourse. MTT test was used to calculate the cell's survival rate. Cells were planted in 96 well plates for this purpose and left to attach for one day before being subjected to the planned studies.

Irradiation setup

After 24 hours of incubation at 37°C, providing enough time for the cells to attach to the surface fully, the cells were cured with plant extract (Capsaicin and Fraxetin), chemotherapy (cisplatin) and radiation in linear accelerator (Elekta Synergy 3630, LINAC, Imam Hassan Al-Mujtaba Hospital, Iraq). Cells were treated with 6 MeV photons and a 3 Gy/min dose rate. The cells were exposed to Individual gross dosages 1 Gy, 3 Gy and 5 Gy with a field size of 10 cm^2 × 10 cm^2. 3 cm thickness of a Perspex sheet was laid on top of the six wells-plate, and three centimetres of a perspex sheet was used under the bottom of the plate as a source of backscatters and Source Surface Distance (SSD) 100 cm photons. After consummation irradiation, the cells were brooded for two days at 37°C in a humidified 5% CO_2 environment. The cells were stabled and stained with 0.4% crystal violet before counting visible colonies with more than 50 cells. The Inhibition rate was calculated based on the survival of the non-treated group, and the survival fraction of the treatment group was obtained by following the formula:

$$\text{Inhibition rate}=\left[\left(\text{O.D}\left(\text{control}\right)-\text{O.D}\left(\text{sample}\right)\right)/\text{O.D}\left(\text{control}\right)100\%\right]$$

MTT Assay

The MTT test was measured by the formerly described [28]. Cells were treated with MTT reagent one to two days after irradiation. The absorbance at 570 nm was determined utilising a microplate reader (Mutiskan EX, Thermo Lab systems). The viability of control (Non-treated cells) was considered 100%.

Statistical analysis

GraphPad Prism 5.0 was used for statistical analysis using one-way ANOVA and Tukey's multiple comparison test. Results were significantly significant ($p<0.05$) compared to the control group.

RESULTS

For Caco-2, capsaicin in conjunction with radiation therapy at a dosage of 1 Gy irradiation resulted in around 59% viable cells, while the pre-treatment with plant extract of capsaicin was established to be more efficacious, departing just 50% viable cells. Different findings were obtained in the amalgamation of capsaicin with the dosages of 3 Gy and 5 Gy (49% and 41% alive cells). Unlike the capsaicin extract, the pre-treatment of Caco-2 by the fraxin extract and chemotherapy (cisplatin), cell viability was around 55% and 39%, but the findings were statistically significant. Different results were found in the combination of fraxetin with the doses of 1 Gy, 3 Gy and 5 Gy (69%, 58% and 46%, respectively, alive cells). Unlike the plant extract, the treatment of Caco-2 by cisplatin and radiation (1 Gy, 3 Gy and 5 Gy), cell viability was about 59%, 49% and 43%, respectively. For the capsaicin extract, the better consequence was beneath radiation of 5 Gy, which resulted in about 59% inhibition. In contrast, for the fraxetin extract of the Fraxinus beneath radiation of 5 Gy, the result was 44% inhibition, as shown in figure 1.

The HDFn seems more responsive to the amalgamation of Capsaicin extract and the 5 Gy radiation dosage (51% alive cells). The fraxetin extract surfaced the lowest inhibition activity, demonstrating viability percentages corresponding to 72%, 65% and 56% when correlated with dosages of 1 Gy, 3 Gy and 5 Gy. For the cells beneath a 1 Gy and 3 Gy radiation therapy, the capsaicin extract also showed low inhibition, resulting in a decrease in the number of live cells by 64% and 59%, respectively. For cells, cisplatin in incorporation with radiation therapy at the doses of 1 Gy, 3 Gy and 5 Gy radiation resulted in approximately 64%, 57% and 54% viable cells, respectively. The findings revealed that the higher cell viability ratio was for fraxetin medications, around 72%, beneath 1 Gy irradiation, and fraxetin 65% when correlated with 3 Gy (Figure 2).

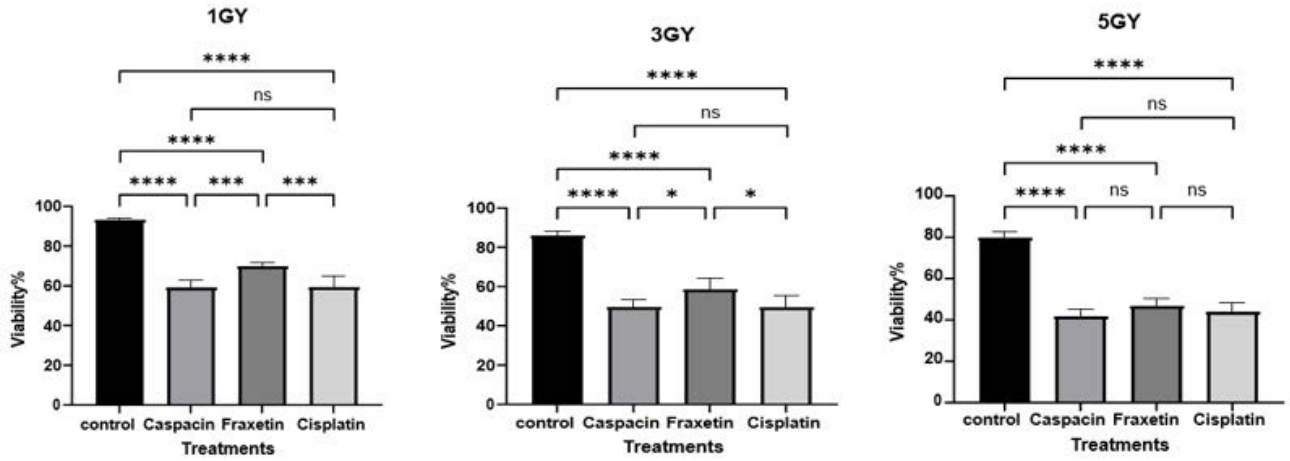

Fig. 1. Viability in Caco-2 is treated with Capsaicin, Fraxetin extracts, and chemotherapy (cisplatin), which is irradiated with 1 Gy, 3 Gy, and 5 Gy

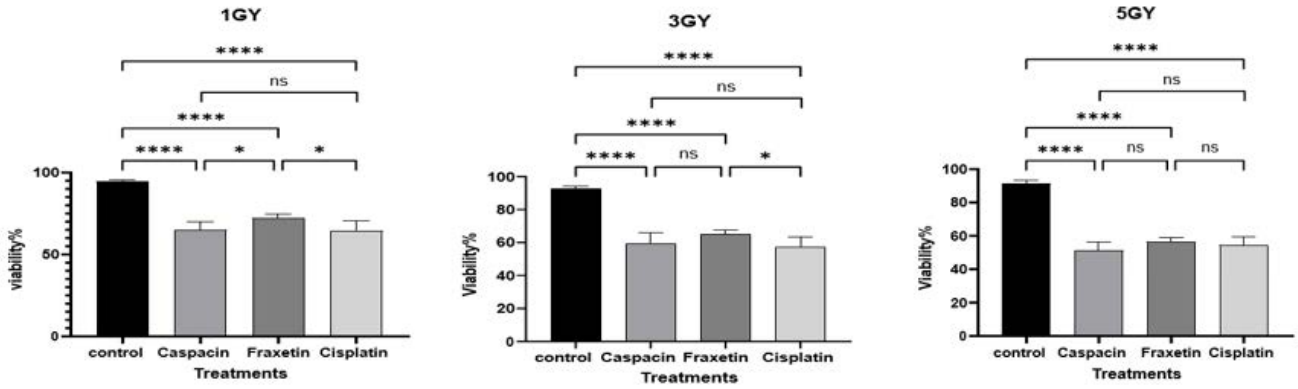

Fig. 2. Viability in HDFn is treated with capsaicin, fraxetin extracts, and chemotherapy (cisplatin), and it is irradiated with 1 Gy, 3 Gy, and 5 Gy

DISCUSSION

Combination treatment consists of the concurrent administration of a traditional chemotherapy medicine (or, in some cases, a radiation program) together with one or more normal bioactive substances, typically of plant or fungal origin with low molecular weight. This amalgamation of anti-cancer medications might be used in tumour cell lines, animal models, or clinical trials [29]. In this research, we epitomise stream knowledge describing varied synergistic impacts on colorectal cancer cell lines and human dermal fibroblasts normal, of natural bioactive (Capsaicin and Fraxetin), which might be significant concerning minimise final dosages of the radiotherapy and chemotherapeutic, although maintaining its impact. Further research demonstrated that capsaicin might suppress cell reproduction by inducing apoptosis in different human tumour cells [30]. Others have reported that capsaicin may stimulate tumour development. There is debate over capsaicin's anti-cancer and carcinogenic impacts [31, 32]. The current study detected the cancer-preventing ability of capsaicin in CRC via a sequence of molecular processes, as shown in the findings.

Incorporating capsaicin with chemotherapy medications or radiation treatment might give techniques for increasing the sensibility of chemotherapeutic or radiotherapy, reducing harmful aspects impacts, overcoming chemotherapeutic resistance, and increasing radiation therapy tolerance. Based on the results, capsaicin might become a supplement to established treatment approaches like chemotherapy or radiation in the near future. Thus, more well-controlled studies and clinical and preclinical tests are needed to confirm CAP's thorough antitumor effect when mixed with other conventional drugs or radiation therapy as efficacious chemotherapy or radiation therapy sensitiser [21]. In the current research, fraxetin showed potential anti-cancer efficacy in CRC. Although fraxetin has been shown to suppress numerous malignancies, this is the first study that surfaced its effectiveness in inhibiting CRC.

According to studies, fraxetin has an anti-cancer impact by reducing the malicious development of cancer. Fraxetin, for example, inhibits the reproduction of human liver cancer cell lines Huh7 and Hep3B and prompts apoptosis through mitochondrial dysfunction [33]. Fraxetin also suppresses Michigan Cancer Foundation-7 (MCF-7) breast cancer cell reproduction and fosters cell death [23]. According to a modern study, fraxetin suppresses glioma cell reproduction and metastasis via inactivating Janus Kinase 2/Signal Transducer and Activator of Transcription 3 (JAK2/STAT3) signalling [34]. Fraxetin appears to be a much more promising natural chemical in anti-cancer treatment. It was discovered that fraxetin suppressed the growth of colon cancer cells (cell lines HCT116 and DLD-1). Its effect was associated with the arrest of the cell cycle in the S phase and the activation of cell apoptosis. The JAK2/STAT3 signalling pathway was shown to reduce p-JAK2 levels following fraxetin administration. Furthermore, fraxetin has a low toxicity [35]. Fraxetin clearly impacted normal human dermal fibroblasts (HDFn) but drastically macer-

ated cell viability in colorectal cancer cell Caco-2. The combination of fraxetin and cisplatin or radiation exhibits an additive interaction, which seems to be a viable technique in CRC treatment.

The toxicology of radiotherapy in cells is dosage-dependent, and it can prompt sublethal destruction sufficient to trigger apoptosis [36]. The incorporation of chemotherapeutics and radiation produces better anti-cancer outcomes than radiotherapy alone. To lessen the aspect effects and toxicology produced by these standard medicines, studies have investigated other sources of therapies, more efficacious and less harmful. Little has been published regarding CRC utilising this novel plant-based method, although promising findings have surfaced. It was recently discovered that plant extracts have a high cytotoxic ability in cell lines of different cancer kinds [37].

This is significant because these treatments may aid in the reduction of adverse effects in individuals receiving traditional chemotherapy and radiotherapy. Also, these molecules might exhibit synergistic effects by numerous cell cycle passageways, including those involved for resistance phenotypes: transcription factor, membrane receptors, adhesion and structural molecules, cell cycle regulatory components, and apoptotic passageways. This study underlines the significant biological ability of capsaicin and fraxetin associated with radiotherapy and chemotherapy for CRC therapy. Here, it was demonstrated that Caco-2 and HDFn treated with capsaicin, fraxetin, and cisplatin presented different percentages of cell viability.

CONCLUSION

The effectiveness of capsaicin and fraxetin extracts was evaluated in combination with chemotherapy and radiation therapy in treating Caco-2 and normal HDFn. The study produced encouraging results and showed the prospect of capsaicin as an additional therapy alongside conventional treatments. In addition to the irradiation with radiation therapy, capsaicin exhibited remarkable cell viability suppression, especially at a dosage of 5 Gy, thus planning its possible use as a sensitizing agent. Yet, discovering that capsaicin may play a part in cancer prevention also raised some controversial issues that required further study. Fraxetin, in addition to its anti-cancer activity in CRC cells, is a novel finding in this work. Its capacity to suppress cancer cell progression and trigger apoptosis indicates that it could be a useful component of current treatment protocols. Additionally, fraxetin's minimal toxicity profile renders it a suitable partner for a cisplatin or radiotherapy treatment. Though plant extracts are as effective as cisplatin when combined with radiation, in cell lines, extracts linked with irradiation showed inhibited activity comparable to or close to the confederation of cisplatin and radiation. This research shows the possible biological significance of some plant extracts when associated with traditional therapy for cancer.

STATEMENTS AND DECLARATIONS

Conflict of interest

Authors have no conflict of interest to declare.

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