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Research Paper

Apoptosis is induced by curcumin in synergism with 5FU in human colorectal cancer cell line.

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ABSTRACT

Background: Curcumin, a phenolic derived from the rhizome of the Curcuma longa linn (*Zingi-beraceae*), has been recognized as a promising anti-cancer drug due to its multiple properties, including anti-inflammatory,antioxidant and anti-angiogenic. Also some evidences show its' anti-cancer effect in some malignancies as in colon, prostate and breast cancer. To show better, the role of curcumin in colon cancer and apoptosis in comparison with 5flurouracil (5-FU) we have planned this project.
Material and methods: For Proliferation test we used MTT assay and for apoptosis study we used TUNEL assay. Also 5FU has been provided as positive control
Results: Our experiment has shown that curcumin, in a dose-dependent manner, reduces cell viability and increases apoptosis in HT29, SW480 and SW742 colorectal cancer cell lines as analyzed by MTT and TUNEL assay, respectively. Moreover, combined with 5FU synergistically increased the number of hypodiploid cells and DNA fragmentation and decreased cell viability in all colorectal cancer cell lines.
This study demonstrates that adding curcumin to 5FU have a synergic anti-mitogenic and apoptotic effect on colorectal cancer cell lines. These results suggest a potential use of serotonin 5-HT_{2A} receptor antagonist in co-treatment with curcumin in colon cancer therapy.

Keywords: curcumin, colon cancer, 5FU, MTT assay, Apoptosis

INTRODUCTION

Colorectal cancer affects one million people annually and is the second most common cause of cancer death in the world [1]. Recently, significant progress has been made in the treatment of this malignancy and new chemotherapeutic agents have been introduced such as 5-Fluorouracil-leucovorin (tymidylate synthase inhibitor), Irinothecan (topoisomerase inhibitor), Oxaliplatin (DNA replication Inhibitor), Bevacizumab (anti-angiogenic), and Cetoximab (monoclonal antibodies targeting the EGFR) [1]. However, although many progress in treatment but chemotherapy is with side effects and long-term treatment failure.

Curcumin, a phenolic derived from the rhizome of the Curcuma longa linn (*Zingi-beraceae*), has been introduced as a promising anti-cancer drug due to its multiple properties, including anti-inflammatory [2], antioxidant [3, 4] and anti-angiogenic [5, 6]. In those areas where curcumin is a staple part of the diet, lower incidence of urothelial malignancies and colorectal cancer has been observed [7-9]. In animals, administration of curcumin inhibits polyp formation and increased cell death in existing colon cancer lesions [10]. Among the potential pathways involved in this anti-carcinogenic properties, inhibition of nitric oxide synthase, activation of receptor tyrosine kinase and protein kinase C (PKC), alteration of the transcriptional factors c-jun/AP-1 and p53 as well as inhibition of arachidonic acid metabolism, lipoxygenase and cycloxygenase activity have been described[4, 11,12]. Combined to others anti-cancer chemicals herbal medicines such as green tea, curcumin also induce a strong inhibitory effect in oral cancer and non-small cell lung cancer [13, 14]. Based on

MATERIALS AND METHOD

these observations, curcumin may be suggested as a potential cancer chemo preventive agent. According to these backgrounds, we planned this research to show better curcumin role in colorectal cancer during an in-vitro study and to show if we can find a synergism effect between this herbal supplement with 5-FU as a routine chemotherapeutic agent.

Materials: RPMI 1640 medium, MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent,5FU(5 Floururacil) and curcumin were purchased from Sigma-Aldrich (St- Louis, MI, US). Fetal calf serum (FCS), L-glutamine, 3, 3 diaminobenzidine (DAB),Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) *in situ* fluorescent detection kit were purchased from Roche Diagnostics (Penzberg, Germany).

Colorectal cell lines and tissues: Colorectal cancer cell lines of three types (HT29, SW480, and SW742) were obtained from the cell bank of the Institute Pasteur of Iran (Teheran, Iran).

MTT assay: Colon cancer cell lines were plated at a density of 1×10^4 cells/well in a 96-well plate in a final volume 100 µl/well. After 24 h at 37°C in 5% CO_2 atmosphere, cells were treated with curcumin at 0, 3.125, 6.25, 12.5, 25, 50 and 100 µM with or without a one hour pre-treatment with 5FU (0.1 µM or 10 µM). Cells cultures with medium only served as a negative control. After 48 h of incubation at 37°C in 5% CO_2 atmosphere, the culture medium was removed and 8 µl MTT reagent (diluted in PBS at a concentration of 4 mg/ml) was added to 50 µl of fresh culture medium at a final concentration of 0.55 mg/ml (final volume 100µl/well). Cells were then incubated for 4 h, the resulting MTT formazan crystals were dissolved with DMSO (50 µl/well) by pipetting up and down 30 times, and the absorbance was spectrophotometrically measured at a wavelength of 570 nm (with a reference wavelength of 690 nm) using a plate reader. Each assay included blank (culture medium without cells) and vehicle control (cells exposed to culture media only). Cell viability (% of control) was determined using the following formula:

$$(A_{(570-690nm)} \text{ of each treated wells} \div A_{(570-690nm)} \text{ average of control wells}) \times 100.$$

TUNEL assay: Apoptosis was evaluated using a commercially available TUNEL kit as previously described [15, 16]. Briefly, colon cancer cells were seeded at a density of 1×10^4 cells/well (final volume 100 µl/well) in 96-well plate. After 24 h, cells were treated with 25 µM curcumin, with or without 10 µM 5FU, for 10 h at 37°C in 5% CO_2 atmosphere, and then were assayed for apoptosis with TUNEL apoptotic procedure. After fixation and prelabeling, cells were washed twice with PBS (200 µl/well) and then suspended in 50 µl/well TUNEL reaction mixture (50 µl of enzyme solution in 450 µl label solution); for the negative control, only 50 µl of label solution was added to the wells. Then cells incubated for 60 min at 37°C in a humidified atmosphere in dark. After this labeling period, the number of TUNEL-positive cells was analyzed by microscope. Apoptosis index was determined as the percentage of TUNEL-positive cells to total cells in a suspension with at least 500 number of tumor cells. Under microscopic area necrotic cells were differentiating from apoptotic cells according to their cellular morphologic changes and loss of nuclear membrane integrity.

Statistical analysis: For MTT assay and TUNEL experiments, data analysis was performed by Student t-test analysis and one-way Anova.

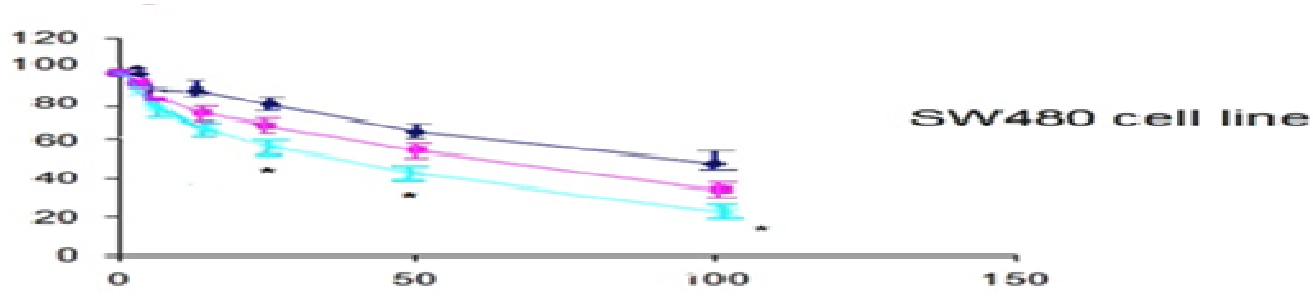
RESULTS

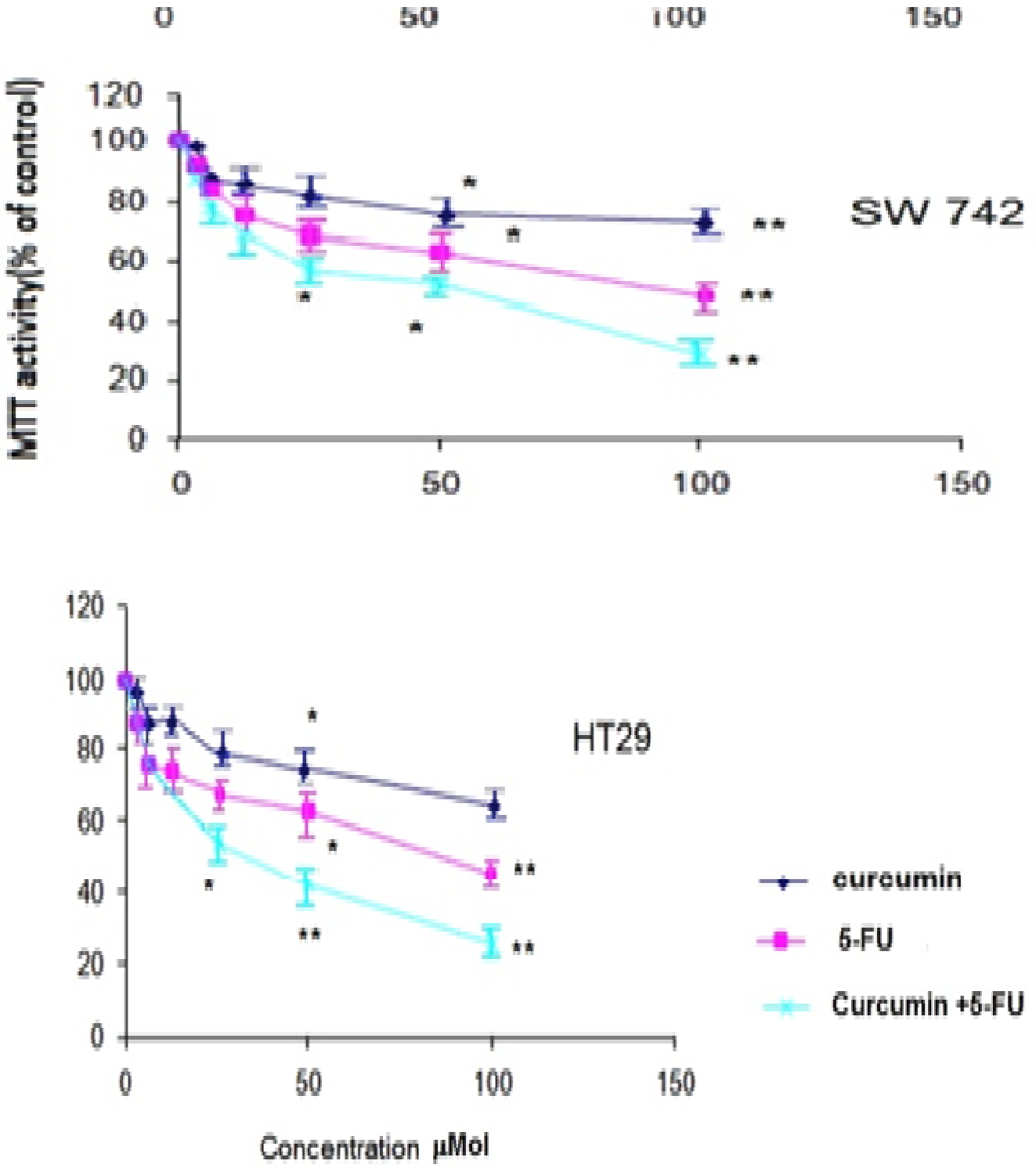
Curcumin in synergy with 5FU decreased colorectal cancer cell lines viability. The effect of curcumin and 5FU individually on HT29, SW480 and SW742 colorectal cancer cell lines viability was first analyzed by MTT assay.

Curcumin alone at 25 to 100 µM reduced cell viability in all cell lines, and this effect is higher in SW480 (40-45%) compared to HT29 and SW742 (10–15%) (Figure 1).

The same profile of reduction in viability was observed with 5FU even slightly more pronounced than with curcumin. The combination of curcumin and 5FU at 25, 50 and 100µM for both drugs, produced a significant synergic reduction of cell growth and viability in all three cell lines.

Figure 1 Effect of 5FU and curcumin, alone and in co-treatment, on HT29, SW480, and SW742 colon cancer cell lines viability.





Legend: Cells were treated for 48 h with increasing concentrations of curcumin, in presence or absence of 10 µM 5FU, and cell viability was determined by MTT assay. The results represent the mean (± SEM) values obtained from three different assays (with three different cell passages) in triplicate. The coefficient of variation is less than 10%. * $P < 0.05$ and ** $P < 0.01$ vs. control determined by Student's t test.

Curcumin in synergy with 5FU induced colorectal cancer cell lines apoptosis. To determine if the effect of curcumin on cell viability was due to apoptosis, colorectal cancer cells lines were treated with 25 µM curcumin alone and in combination with 25 µM 5FU and analyzed by the TUNEL assay and fluorescence microscopy. Figure 2 show that curcumin induced SW480, SW742 and HT29 colorectal cancer cell apoptosis

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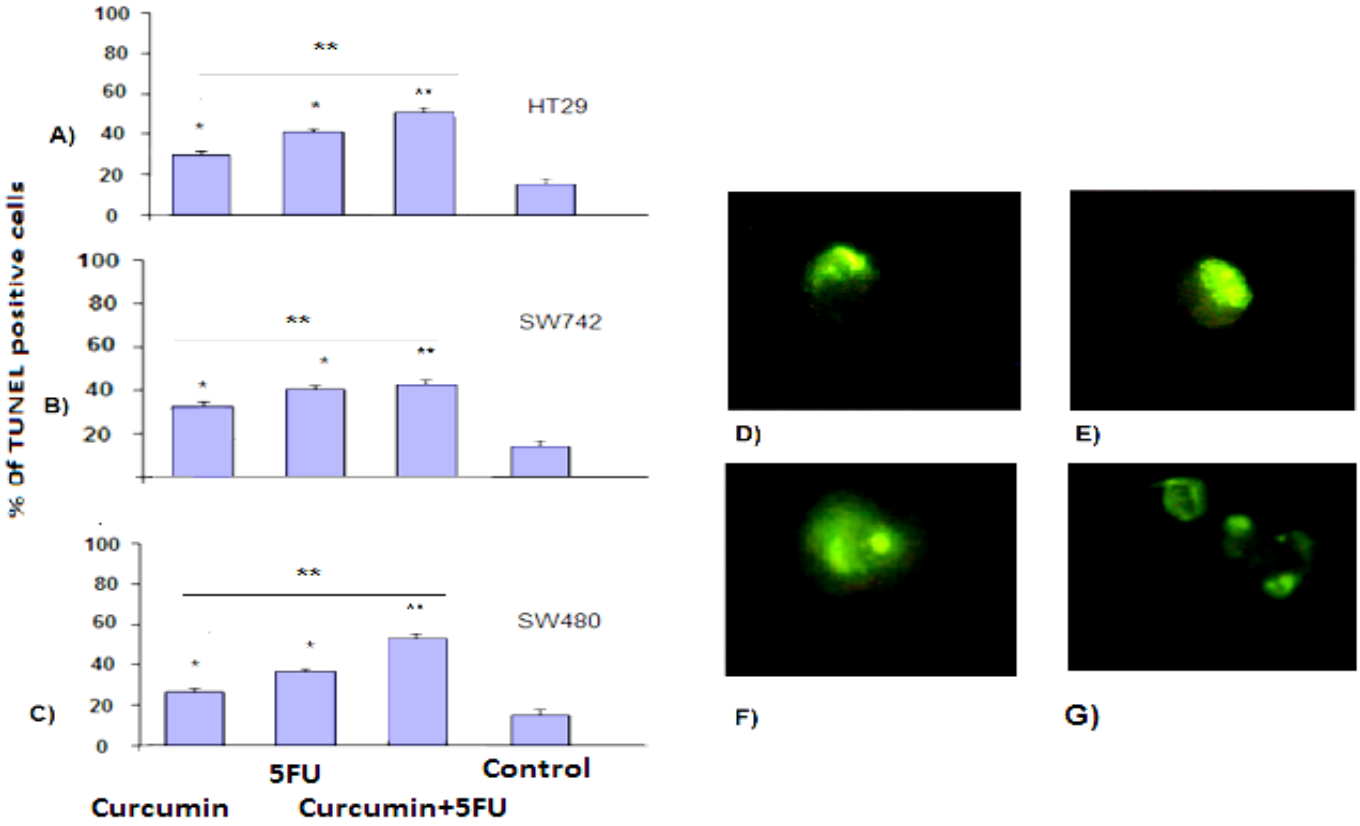
Figure 2 Effect of 5FU and curcumin, alone and in co-treatment, on HT29, SW480, and SW742 colon cancer cell lines apoptosis.



Legend: (A) HT29, (B) SW742, and (C) SW480 colon cell lines were treated with 25 µM of curcumin, with or without 10 µM 5FU (cur + 5FU) or with media only (control), for 10 h at 37 °C in 5% CO 2 atmosphere, and then were assayed for apoptosis with TUNEL apoptotic kit (Roche). TUNEL-positive cells were analyzed by microscope and express as the percentage of apoptotic cell to total cell. * $P < 0.05$ and ** $P < 0.01$ vs. control determined by Student's t test.(D) Apoptosis induced by curcumin (E) 5-FU (F) Curcumin+5FU (D) Necrosis induced by curcumin in concentration more than 25 µM. At concentration less than 25 µM we observed low grade of apoptosis and at higher concentration the cells had gone toward necrosis. Also curcumin combined with 5FU markedly enhanced (about 2 fold) colorectal cancer cells lines death compared to the curcumin or 5FU alone in all cell lines (Figure 2 A-C).

DISCUSSION

5Flurouracil (5FU) is a tymidilate syntethase inhibitor and anti-metabolite drug which its' use as anti-cancer chemotherapy has been confirmed routinely in colorectal cancer [1] It has also synergic effect with Leucovorin (Folinic acid) for this purpose [1] .

Our results showed that curcumin which is phenolic derived from the rhizome of the Curcuma longa linn has anti-prolifertative and apoptotic effect on colorectal cancer cell line.

Interestingly, curcumin in combination with 5FU caused two fold enhanced cell growth reduction and induce death in cells at concentrations that each drug alone is less effective. So we can suggest a synergistic effect between curcumin and 5FU in colorectal cancer chemotherapy.

Dietary constituents have been revealed to provide protection against many diseases [3, 8] . It has been reported that curcumin, inhibit the clonogenic growth and induce apoptosis of several human and murine leukemia cell lines [8, 9]. Indeed, curcumin is frequently used as anti-cancer drugs and has been demonstrated to sensitize cancer cells to chemical therapy [17]. This chemical induced its effects by blocking the cell cycle and the up-regulation of anti-oncogene such as suppressor tumor p-53[9, 11]. Here, we have shown that combination of curcumin with 5FU pronounced the reduction in growth rate of colorectal cancer cells and enhanced the apoptosis rate. This is the first study which showed a synergism in increasing of apoptosis and reducing of proliferation between curcumin and 5FU in colorectal cancer cell line and shows a benefic effect for curcumin in colorectal cancer chemotherpy. Off-course some other complementary in-vivo studies are necessary to show better the cellular and molecular aspects of curcumine mechanism in chemotherapy.

CONCLUSION

In summary, this study suggest that combination of curcumin with 5FU may be an alternative to improve chemotherapy of colorectal cancer and with adding a supplement without any serious side effects or toxicity may be useful in lowering the dose of chemotherapeutics and to treat better the cancer disease.

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