ISSN: 03875547
Volume 45, Issue 01, February, 2022

Measurement of Carcinoembryonic Antigen Activity (CEA) after cellular response to anticancer agents.

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Keywords:
5-fluorouracil, feboxustat, curcumin, LS174T cell line, cytotoxicity and Carcinoembryonic antigen (CEA)

ABSTRACT

The results of numerous studies have revealed that cancer cell growth has been affected in response to treatment with anticancer agents, also some cancer related antigen were affected (decrease or increase) as a results when the cancer cell were treated with anticancer agents, in the present study, the in vitro cytotoxicity effects of different drug (5-fluorouracil, curcumin and feboxustat) was measured by using crystal violet technique, the results showed significant ($p \leq 0.001$) anticancer activity on LS174T colorectal cancer cell line at different concentrations. Also for the same drugs (5-fluorouracil, curcumin and feboxustat) Carcinoembryonic antigen (CEA) level was measured by using ELISA technique, the results showed significant ($p \leq 0.001$) decrease in CEA activity at different concentrations. Study the cytotoxic effect of effect of 5-FU, Curcumin and Febuxostat on colorectal cancer LS174TCell Line, Study the effect of 5-FU, Curcumin and Febuxostat on the level of Carcinoembryonic antigen (CEA expression). 5-FU, Curcumin and febuxostat when used, showed good anti-cancer activity on colorectal cancer LS174T cell line. On CEA expression, the 5-FU, Curcumin and febuxostat when used, cause a decrease in the expression of CEA levels suggesting tumor shrinkage and anti-cancer effect.

1. INTRODUCTION

Colon cancer typically affects older adults, although it can happen at any age [1]. In case of development of colon cancer, a number treatments are offered to help control it, like surgery, radiation therapy and drug treatments, like chemotherapy, targeted therapy and immunotherapy [2]. The LS174T epithelial cell line was isolated from a 58-year-old Caucasian female with colorectal adenocarcinoma and produces high amounts of carcinoembryonic antigen (CEA) [3]. 5-fluorouracil (5-FU) is widely used in the treatment of cancer. Over the past 20 years, increased understanding of the mechanism of action of 5-FU has led to the development of strategies that increase its anticancer activity. Despite these advances, drug resistance remains a significant limitation to the clinical use of 5-FU but remains the most widely used agent in the treatment of colorectal cancer [4]. Curcumin is a polyphenol derived from the herbal remedy and dietary spice turmeric. It possesses

diverse anti-inflammatory and anti-cancer properties following oral or topical administration, For numerous decades ago, plant-extracts and their active derivatives have long been considered to be promising candidates to treat a variety of human diseases such as infections, numerous kinds of skin lesions and so on; however, such natural products and/or their synthetic analogues may induce a spectrum of adverse effects [5]. Because of these natural products in general speaking exhibit less toxicity than synthetic compounds, they have been the target materials of growing research interest especially for the management of cancer and its subsequent complications [6]. The 3rd agent used was Febuxostat and It`s a medication used long-term to treat gout due to high uric acid levels is effective for the prevention of hyperuricemia accompanied by tumor lysis syndrome (TLS) during cancer chemotherapy [7], [8]. The in vitro experiments carried out on human colorectal carcinoma cells clearly demonstrated as the encapsulation of the febuxostat into the drug delivery system, significantly improved all the parameters related to the toxic potential of the drug towards cancer cells, including the IC50 decrease, the enhancement of anti-proliferative activity, the increase of the percentage of apoptotic and necrotic cell populations paralleled by an increment of intracellular caspase−3 concentration, and, finally, the decrease of Matrix Metalloproteinases in cancer cells [8]. Carcinoembryonic antigen is a 180–200 kD glycosylated protein. It is the most useful tumor marker to distinguish between benign and invasive carcinomas of the colon as benign adenomas do not because an increase in CEA levels [9]. Study the cytotoxic effect of effect of 5-FU, Curcumin and Febuxostat on colorectal cancer LS174TCell Line, Study the effect of 5-FU, Curcumin and Febuxostat on the level of Carcinoembryonic antigen (CEA expression).

2. Materials and Methods

2.1 Chemicals

5-florouracel, febuxostat and curcumin from Sigma-Aldrich. Dimethyl sulfoxide (DMSO) from Roth (Germany), Phosphate buffer saline(PBS) packets from BioPLUS (USA) RPMI 1640 medium w/L-glutamine, 25mM HEPES (powder) from Gibco (UK)

2.2 Stock solution preparation

2.2.1 Preparation of 5-FU stock solution

Five-FU is white crystalline powder which is sparingly soluble in water with solubility 1gm in 80ml water, we dissolve 10 mg of 5-FU in 1ml of DDW to prepare stock solution of 10000µg/ml.

2.2.2 Preparation of febuxostat stock solution

Febuxostat is a white crystalline powder It is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide. In our experiments we use DMSO as a solvent to prepare 1000µg/ml by dissolving 1mg febuxostat in 1 ml DMSO.

2.2.3 Preparation of curcumin stock solution

Curcumin has Orange-yellow, crystal powder which is Insoluble in water, Insoluble in ether; soluble in alcohol and DMSO. In our experiments we use DMSO as a solvent to prepare the 1000µg/ml stock solution by dissolving 1mg curcumin in 1ml DMSO

2.2.4 Preparation of LS-174T Cell Line for Cytotoxicity Assay

LS-174T cell line in the frozen vial obtained from tissue culture laboratory in the College of Medicine / University of Babylon. growth was maintained in a 25 ml culture flask, with a complete growth medium containing 10% FBS and antibiotics and incubated at 37˚C, after that thawing of LS174T cell line was done, then Harvesting and Sub-Culturing of LS174T Cell Line. Finally 96-well plates were seeded with human colorectal carcinoma LS-174T cells in a seeding density of 5*105 that were prepared for treatment with 5-

ISSN: 03875547
Volume 45, Issue 01, February, 2022

FU, Febuxostat and Curcumin.

2.3 Cytotoxicity Assay

The 96-well plates were seeded with human colorectal carcinoma LS174T cells in a seeding density of 5*105 and the wells of the plate (except one column from each plate which left without treatment as a control), exposed to 200 µL of six serial dilutions of 5-FU as follow (1500, 750, 375, 187.5, 93.75 and 46.875 µg/ml). After the end of the experiments, the plate was covered with the plastic lid and incubated for 24 hours. The same procedure repeated but by use febuxostat with concentrations (200, 100, 50, 25, 12.5 and 6.25µg/ml) and curcumin with concentrations (50, 25, 12.5, 6.25, 3.12, 1.56 µg/ml). Afterward, the plates were washed with 200 µl of a sterile PBS after collecting the supernatant for CEA kit testing, and the effect of the 5-FU, Febuxostat and curcumin on LS174T cell line growth were assessed by crystal violet cytotoxicity assay.

2.4 Carcinoembryonic Antigen Activity (CEA) kit

Depending on procedure that found with CEA kit from Elabscience USA, for all agents used in the experiment. Statistical Analysis: All data were collected and analyzed by Microsoft Office Excel 2010 and Sigma plot version 13 software. ANOVA test was used to assess significant differences among the means of data, where the p-value ($p \leq 0.001$, $p \leq 0.05$) were considered to be statistically significant.

3. Results

3.1 Cytotoxicity Assay

3.1.1 Cytotoxic effect of 5-FU on LS174T cell line.

The results showed that there was a significant ($P \leq 0.001$) decrease in the viability percentage of LS174T colon cancer cell line for all concentrations in comparison to the control group. (Figure 1)

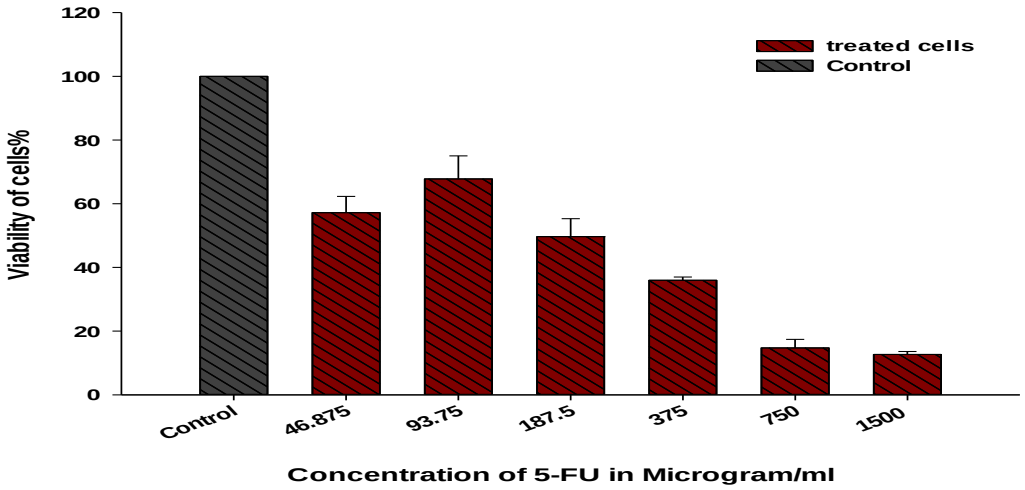

Figure 1. Cytotoxic Effect of 5-FU on LS174T Cell Line after Incubation for 24 Hours.

3.1.2 Cytotoxic effect of febuxostat on LS174T cell line

The results showed there was a significant ($p<0.001$) decrease in viability on LS174T colon cancer cell line at all concentrations used when treated with different concentrations of febuxostat after incubation period of 24 hours. (Figure 2)

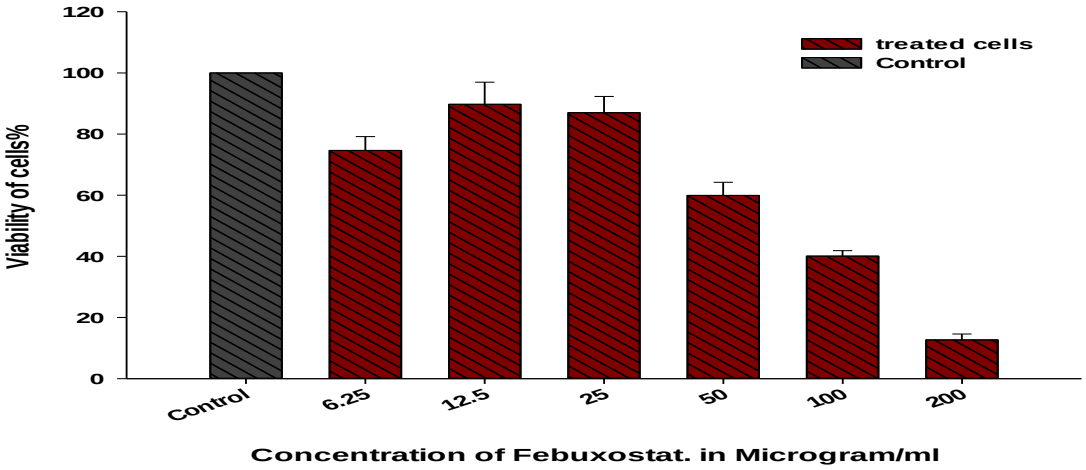

Figure 2. Effect of febuxostat on LS174T Cell Line after Incubation for 24 hr.

3.1.3 Cytotoxic effect of curcumin on LS174T cell line

The results showed that there was a significant decrease (P ≤0.001) in the viability percentage of LS174T colon cancer cell line in all concentrations used except at 1.56 µg/ml which showed no significant (P = <0.001) decrease in the viability as shown in (Figure3)

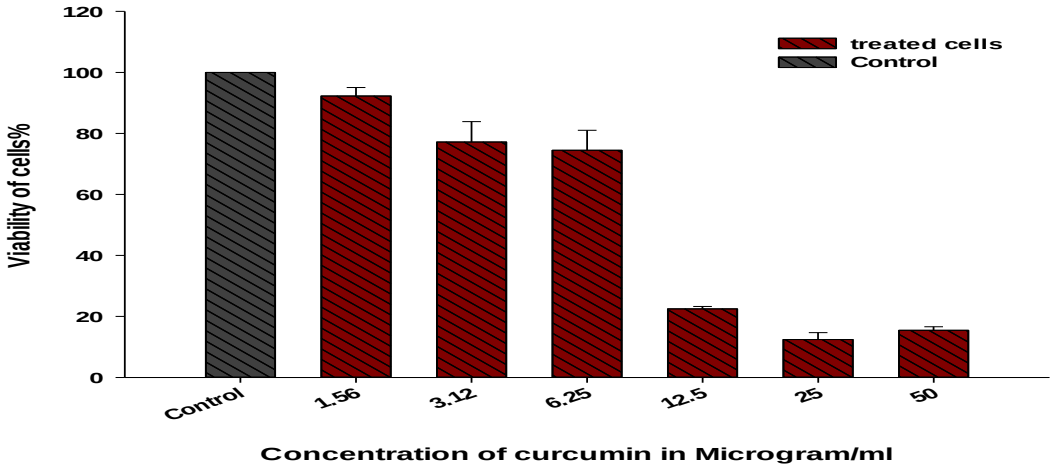

Figure 3. Effect of curcumin on LS174T Cell Line after incubation for 24 Hours.

3.2 Carcinoembryonic Antigen kit Results

3.2.1 The effect of 5-FU on CEA expression

The results showed that there was significant decrease (P = <0.001) in the carcinoembryonic antigen (CEA) expression when using 5-FU at three different concentrations (1500 µg/ml, 375 µg/ml and 46.875 µg/ml) in the colon cancer LS174T cell line. (figure 4)

ISSN: 03875547
Volume 45, Issue 01, February, 2022

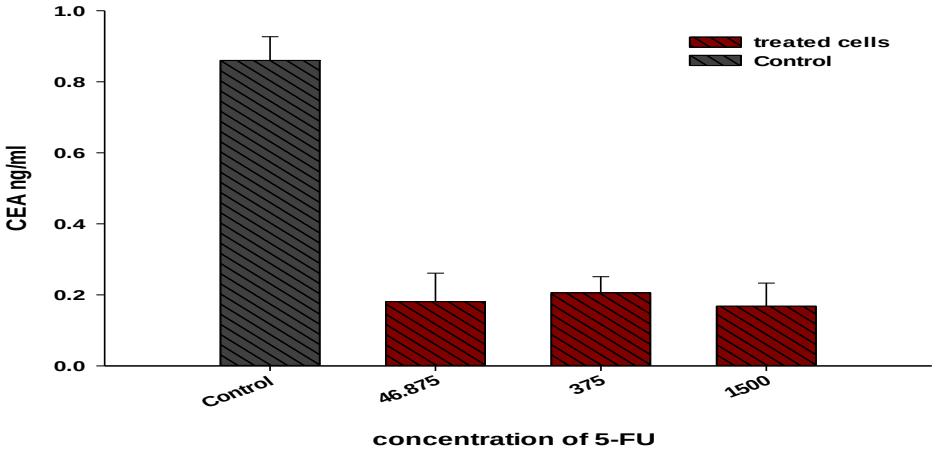

Figure 4: the effects of 5-FU on CEA expression

3.2.2 The effect of Febuxostat on CEA expression.

The results showed that there was significant decrease (P = <0.001) in the carcinoembryonic antigen (CEA) expression when using Febuxostat at three different concentrations (200 µg/ml, 50 µg/ml and 6.25 µg/ml) in the colon cancer LS174T cell line. (figure5).

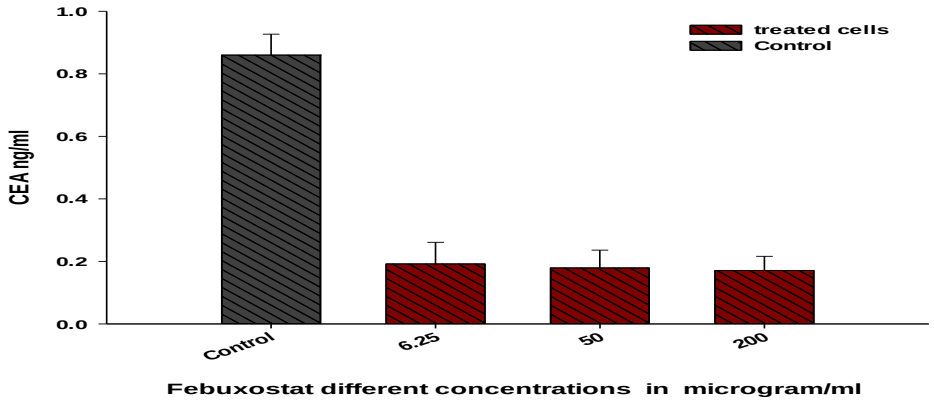

Figure 5 The effect of Febuxostat alone on CEA expression

3.2.3 The effect of curcumin on CEA expression.

The results showed that there was significant decrease (P = <0.001) in the carcinoembryonic antigen (CEA) expression when using curcumin three different concentrations (50 µg/ml, 12.5 µg/ml and 1.65 µg/ml) in the colon cancer LS174 cell line. (figure6).

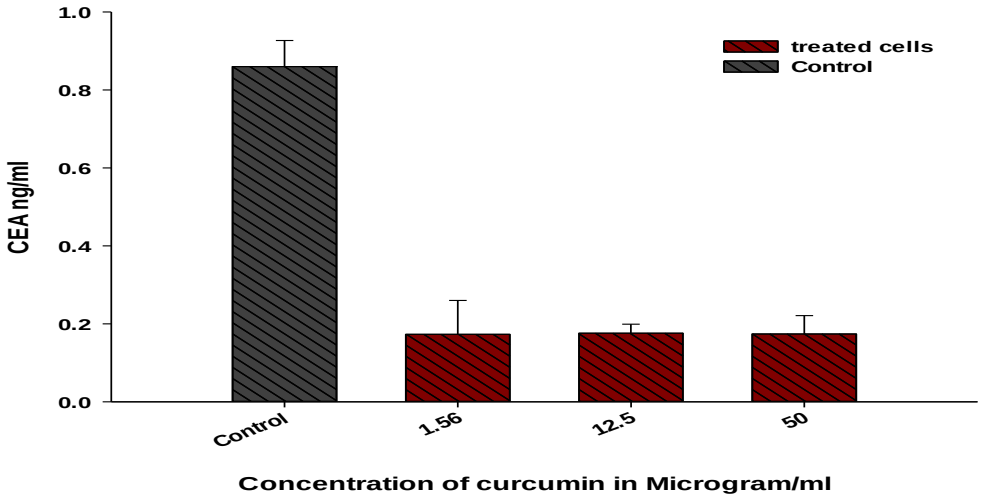

Figure 6: The effect of curcumin alone on CEA expression.

4. Discussion

4.1 Cytotoxicity Assay

The effects of 5-FU, Febuxostat, Curcumin as shown in figures (1, 2 and 3) on the viability of colon cancer LS174T cell line. A study by (Pardini et al., 2011) showed that treatment with 5-fluorouracil (5-FU) is known to improve survival in various cancers. The largest impact of the drug has been reported in colorectal cancer. Active metabolites of 5-FU disrupt both DNA and RNA synthesis through a mechanism involving the folate metabolic pathway. a study by [10] demonstrate for the first time that curcumin, a naturally occurring anti-inflammatory agent and antioxidant, given as a dietary supplement during promotion/progression period still inhibits tumor genesis in the colon, suggesting that administration of curcumin may retard growth and/or development of existing neoplastic lesions in the colon. This also suggests the potential usefulness of this agent as a chemo-preventive agent for individuals at high risk for colon cancer development, such as patients with polyps. also, Curcumin has been reported to modulate growth factors, enzymes, transcription factors, kinase, inflammatory cytokines, and proapoptotic (by up regulation) and antiapoptotic (by down regulation) proteins. This polyphenol compound, alone or combined with other agents, could represent an effective drug for cancer therapy. In colorectal cancer, curcumin exhibited its therapeutic action by affecting several cells signaling pathways. Curcumin inhibited DMH (1,2-Dimethylhydrazine)-induced rat colorectal carcinogenesis and the growth of the in vitro cultured HT 29 cell line by suppressing the PPARγ signal transduction pathway [11]. In addition, curcumin also suppressed the expression of cyclooxygenase-2 (COX-2), and pre-mRNA processing factor 4B (Prp4B) [12], [13]. The AMP-activated protein kinase (AMPK) pathway has gained more interest as an important pathway involved in cancer control. Curcumin has been reported as an inhibitor of colorectal cancer invasion by means of AMPK-induced inhibition of NF-κB, urokinase-type plasminogen activator (uPA) activator, and matrix metalloproteinase-9 (MMP9) [14]. Curcumin has been reported as an agent able to prevent colorectal cancer proliferation by blocking the cell cycle and accelerating apoptosis. It exerted this action affecting thymidylate synthase and its transcription factor E2F-1. This effect caused cell cycle inhibition via downregulation of NF-κB and other survival pathways [15]. Besides, curcumin downregulated the kinase CDK2, leading to the G1 cell cycle. In human colon cancer cells, curcumin significantly inhibited cell growth. Further, it also inducted apoptosis through a mitochondria-mediated pathway.

In human colorectal cancer HCT116 and HT29 cells, curcumin downregulated the expression and activity of hexokinase II (HKII) in a concentration-dependent manner and induced dissociation of HKII from mitochondria, resulting in mitochondrial-mediated apoptosis [16]. The idea for use febuxostat on cancer cell line emerge from the activity and use of this drug in case of tumor lyses syndrome (TLS). Tumor lysis syndrome (TLS) is an oncological emergency that can occur following the treatment of hematologic cancers, and can result in life-threatening complications. It is caused by the rapid destruction of tumor cells and the release of their contents into the systemic circulation, which can cause acute kidney injury, arrhythmias, seizures, and even death [17]. A recent study of adult patients with hematologic malignancies at intermediate to high TLS risk showed that febuxostat was significantly more effective than allopurinol in controlling serum UA level as a means of TLS prevention [18]. Also febuxostat has good anticancer activity on A549 non-small cell lung cancer cells [19]. Recently, several studies have been conducted to examine the febuxostat anti-cancer activity, with particular regard to its ability to enhance cancer cells death via apoptosis and decrease the chemotherapy resistance, then representing a promising candidate for cancer treatment. the enhancement of anti-proliferative activity, the increase of the percentage of apoptotic and necrotic cell populations [8]. Studies suggest that 5-Fluorouracil (5-FU) can activate p53 by more than one mechanism: incorporation of fluorouridine triphosphate (FUTP) into RNA, incorporation of fluorodeoxyuridine triphosphate (FdUTP) into DNA and inhibition of thymidylate synthase (TS) by fluorodeoxyuridine monophosphate (FdUMP) with resultant DNA damage, also the 5-FU metabolite FUTP is extensively incorporated into RNA, disrupting normal RNA processing and function. Significant correlations between 5-FU misincorporation into RNA and loss of clonogenic potential have been shown in human colon and breast cancer cell lines [20]. Studies demonstrated that CEA is not always accompanied by shrinkage of cancerous cells, The sensitivity of falls in CEA levels in the prediction of true responders was 72%. Additionally, the sensitivity of elevated serial CEA levels for the prediction of progressive disease was 81%. Therefore, it should be kept in mind that both an overestimation of tumor response and an underestimation of progressive disease might occur when chemotherapeutic response is assessed by change in CEA levels Furthermore, measurement of CEA levels may be helpful in determining the prognosis of patients with metastatic colorectal cancer receiving chemotherapy [21].

Change in CEA levels in the prediction of progressive disease is more precise than in the prediction of responders. It is clear that CEA levels cannot replace imaging studies in the assessment of chemotherapeutic efficacy, a fall in CEA level is indeed associated with prolonged survival reflecting shrinkage of cancerous cells [21]. One of the most important tumor suppressors in CRC is P53, dictates the sensitivity to 5-FU-based therapies. In addition to its well-known transcriptional activities controlling the expression of a variety of genes important in anti-cancer effects, its crosstalk with other signaling pathways in various cellular processes has been identified [22]. A study by [23] found that a decrease in CEA was associated with tumor response and a CEA increase was associated with disease progression, thus using chemotherapy such as 5-FU can cause decrease in the CEA levels due to shrinkage of cancerous cells. A study by [24] disagrees with our study in which the CEA levels are increased in response to 5-FU chemotherapy due to resistance to chemotherapy that suggests that the selective resistance to 5-FU cytotoxicity was a direct effect of the elevated CEA expression [24]. Tumor lysis syndrome (TLS) is a life-threatening oncological emergency, in which control of serum uric acid (S-UA) levels is important. S-UA-lowering efficacy of a new xanthine oxidase inhibitor, febuxostat [25]. A study showed that Curcuma extract might be associated with a dramatic decrease in the level of carcinoembryonic antigen (CEA), a venous tumor marker [26].

5. Conclusion

5-FU, Curcumin and febuxostat when used, showed good anti-cancer activity on colorectal cancer LS174T cell line. On CEA expression, the 5-FU, Curcumin and febuxostat when used, cause a decrease in the

expression of CEA levels suggesting tumor shrinkage and anti-cancer effect

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