

Cytotoxic Effect of Cassia Fistula Extracts Combined with Anticancer Drug on Human Colon Cancer Cells

Rana Talib Al-Muswie¹, Dheyaa Khalf Al-Omer² and Rana A.Ghaleb³

¹Department of Basic Science- College of Dentistry/University of Thi-Qar

²Department of Medicine-College of Medicine/ University of Thi-Qar

³Department of Human Anatomy-College of Medicine / University of Babylon. Iraq

Email: rana-almusawie@utq.edu.iq

Email: rana.a.ghaleb@uobabylon.edu.iq

Abstract—According to scientific studies, combining particular antioxidants in set quantities with some chemotherapy treatments may increase therapeutic efficacy or lessen the severity of side effects. A pleiotropic phytochemical from the Fabaceae family is called cassia fistula. Although, The current study evaluated the anticancer properties of Cassia fistula in combination with Cisplatin on the colorectal cancer cell line LS47T. LS47T colorectal cancer cells were exposed to C. f. extract, and the results showed that this drastically decreased cell viability in a dose and time dependent manner. Additionally, cells treated with 480 g/ml of Cassia fistula extract had lower viability. Treatment with 120 or 240 mg/kg C.f extract reduced tumor growth, according to in vivo experiments. Due to the study's results, cisplatin alone did not have the same therapeutic impact on colorectal cancer as a combination of plant extract and cisplatin. are required to determine the chemical makeup of the active substances. The findings of this study could aid in the creation of innovative and effective methods for treating colon cancer in humans.

Index Terms— Cell line LS47T ,colon cancer, cisplatin, Cassia Fistula.

I. INTRODUCTION

Cancer-related diseases are now a significant burden and a leading cause of death worldwide, particularly in developing nations. Even in technologically sophisticated nations, cancer has surpassed other diseases as the leading cause of ill health and mortality due to changes in lifestyles, eating habits, and access to curative treatment for many infectious diseases. The development of colorectal malignancies, which are now more accurately detected and categorized using a number of criteria, has a number of causes. This form of cancer is now being successfully treated using a variety of medicines and prognostic indicators(1). Effective treatments for managing different types of cancer, such as chemotherapy, radiotherapy, and surgery, are pricy, disfiguring, and linked to major side effects. These therapeutic approaches are linked to persistent morbidity as well as relapses that happen often. Due to significant side effects and the emergence of resistance, chemotherapy's effectiveness has been restricted (2;3).

Therefore, there is no consistent increase in overall survival from conventional treatment. Therefore, more treatments are required to address the issue of selectivity and drug resistance

in patients with colon cancer. Natural compounds are thought to have high efficacy and low toxicity anticancer activity(4). The effects of an aqueous extract of Cassia fistula seeds on human colon cancer (LS47T) were investigated.

The fruit and seeds of Cassia fistula Linn. (Fabaceae) have been used in traditional medicine and have been shown to offer a number of pharmacological benefits. We tested C. fistula fruit and seed extracts for their ability to inhibit the growth of human colon cancer (LS47T) and breast cancer (MCF-7) cell lines.

II. MATERIALS AND METHODS

A. Preparing of Colon Cancer LS47T Cell line

The colon cancer cell line LS47T was isolated from the primary tumor of colon cancer, was provided by the Iran Center for Cancer and Medical Genetics Research (ICCMGR).

RPMI 1640 (Sigma), 10% fetal bovine serum, 2 g/L sodium bicarbonate and Penicillin-Streptomycin (10,000 U/mL) were used to culture the cells in a flask of 75 cm². Cell monolayers were grown in culture flasks at 37°C after 24 hours incubation period in a 5% CO₂ incubator.

B. Chemotherapy Reagents (Cisplatin)

Cisplatin was purchased from the pharmacy and kept at 4 °C in a concentration of 50mg/50mL.

C. Preparation of Cassia fistula Aqueous Extract

Dry fruits and seeds of the Cassia fistula plant, the subject of the study, were gathered. The plant was then cleaned with distilled water and allowed to air dry. The extraction was carried out as an aqueous extract in accordance with (5), using 20 g of plant powder by weight, 100 ml of boiling distilled water, and 30 minutes of waiting. then left the beaker on the heater with a continuous magnetic stirrer overnight. Next, the extract was centrifuged with 2500 rpm for 10 minutes. The extract was then passed through Whatman filter paper and left overnight. Finally, it was placed in an oven set to 40 to 45 degrees. The final stock concentration (1000 g/ml) was made by diluting weight extract in an appropriate amount of water, and it was then sterilized by passing it through a 0.22 m Millipore filter.

D. MTT test

With a few minor modifications, the MTT analysis described in method (6) was utilized to assess the effect of plant extract on LS174T cells. In this experiment, cells were seeded in 96-well microplates at a density of 5×10^3 cells per 0.1 mL and allowed to adhere. After 24 hours, cells were exposed to the various concentrations of the extract using the serial dilutions method. After a total of 24 hours, 10 μ L from (MTT) was added to each well. After then, the cells were given another three hours of incubation. Following that, 50 μ L of dimethyl sulfoxide (DMSO) were used to dissolve the intracellular MTT formazan. The absorbance was measured at 570 nm using a microtiter plate reader. The calculation of cell viability was achieved using the equation: $(OD_{570} \text{ of the treated sample} / OD_{570} \text{ of the untreated control}) \times 100$.

E. Statistical Analysis

The results of each analysis were collected in triplicate and are represented as means standard deviation (SD). The (SPSS) v.20 application and Student's t-tests were used to conduct the statistical study. It was determined that the results were statistically significant if the p-value ≤ 0.05 .

III. RESULT

Based on its ability of interacting with living cells, the MTT assay transforms a yellow tetrazolium dye into a purple formazan result. The purpose of the current study was to employ the MTT assay to assess how the Cassia fistula extract affected cell growth and proliferation. Figure 1 displays the results from an analysis of all the cells that had been exposed to Cisplatin at different concentrations (0–480 μ g/mL) over the course of 24 hours. As Cisplatin concentration increased, a decline in cell viability was observed. In a concentration-dependent function, the drug-treated cell's survival percentage was significantly lower than the untreated cell's ($P < 0.05$). Results indicated that extracted cells showed lower levels of cell viability than untreated cells (Fig. 2). Against the colon cancer cell line SW480, plant extract doses of 480, 240, and 120 μ g/mL resulted in the highest decrease in the cell viability percentage. The test revealed that the extract of Cassia fistula had no impact on cell line viability at concentrations of 20, 40, and 60 μ g/mL. Cisplatin IC₅₀ was 43.5 μ g/mL. The colon cancer cell line (Figure 3) was treated with cisplatin at a dosage of 480 μ g/mL, which resulted in the highest decrease in the cell viability.

After 24 hours of incubation, the effects of a treatment combining an equivalent dosage of cisplatin and cassia fistula were significant ($p \leq 0.05$) decreased as compared to the control group (cancer cells not receiving treatment) in all concentrations (60, 120, 240, and 480 μ g/mL) as represented in Figure 4. Additionally, (Figure 5) shows the inhibitory concentrations following Cisplatin treatment and Cassia fistula extract at various doses. A decrease in cell viability was seen as the combination's concentration was increased. The survival rate of colon cancer cells after treatment with combination was significantly lower than the untreated cell ($P < 0.05$). High

concentrations of the combination (120, 240, and 480 μ g /ml) demonstrated a growth-inhibitory effect on the colon cancer cell line LS47T.

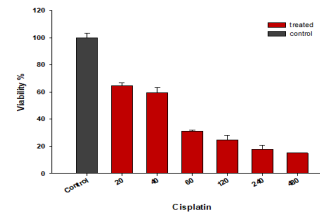


Figure 1 A: Effect of cisplatin on cell proliferation of LS47T cell line.

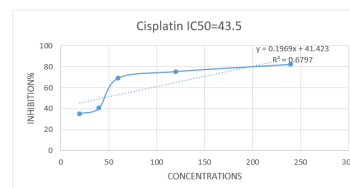


Figure 1.B: The half maximal concentration (IC₅₀) of Cisplatin on colon cancer cell line.

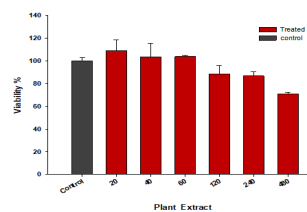


Figure 2: Effect of Cassia Fistula on cell proliferation of LS47T cell line.

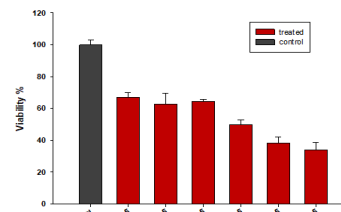


Figure 3 : The effect of IC₅₀ of cisplatin in combination with C. fistula on the LS47T cell line.

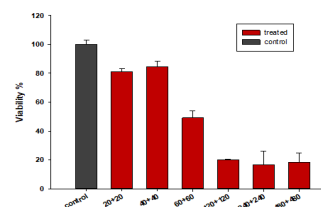


Figure 4 :Antiproliferative activity of equal concentrations of cisplatin with C. fistula on LS47T cell line.

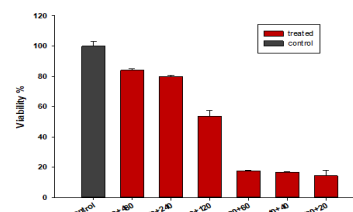


Figure 5: Antiproliferative activity of different concentrations of cisplatin in combination with C. fistula.

IV. DISCUSSION

Patients who receive conventional therapy for colon cancer experience severe adverse effects(7). Alternative therapy approaches with less side effects are desperately needed. Flavonoid-rich fruits and vegetables are frequently advised to lower the risk of cancer(8). There are some plants that are being looked at as potential cancer therapeutics(9). It was found in the current study that the plant extract of Cassia fistula combined with cisplatin might act against colon cancer cells in a synergistic manner and limit proliferation.

After receiving combination therapy, colon cancer cells' survival rate was significantly lower than that of untreated cells ($P \leq 0.05$) in concentration-dependent function. The proof for this was the reduced survival rate. The cell viability experiment revealed that extract inhibited cell proliferation in LS47T cells (Figure 1). Cells from human colorectal cancer were only marginally reduced in their long-term proliferation due to Cassia fistula.

However, according to (10), TCS is active when trachelogenin, the TCS aglycone, is used to treat human colorectal cancer cells. Arctigenin, which has a structure similar to TCS, demonstrated a stronger anti-proliferation effect in mouse-derived CRC cells than in human CRC cells(11), which is consistent with our findings. As a result, some lignans are more potent against murine CRC cells than they are against human CRC cells.

At high doses, the combination prevented the colon cancer cell line SW480 from proliferating. conducted a different investigation to evaluate the anticancer effects of various Cassia fistula fruit extracts on cell lines representing human cervical cancer and breast cancer. Their findings showed that seeds and pulp decreased the activity of nine enzymes, up-regulated the p53 and Bax genes, down-regulated the Bcl-2 gene, and repressed two cancer cell lines. Furthermore(12), found that Rhein suppressed cancer cell growth in a dose-dependent manner in a variety of malignant cell lines, including hepatocellular carcinoma (HepG2), human cervical cancer (SiHa), and breast adenocarcinoma (MCF-7).

DNA damage may also be the cause of the process behind the extract's induction of cell-specific toxicity. Although cellular transformation is often the outcome of unchecked cellular growth caused by changes in numerous cellular pathways, various malignancies are unique in their own ways because they contain their own particular set of mutations(13).

Adenomatous polyposis coli (APC), deletion of p53, and a deficiency in the long arm of chromosome 18 caused by loss of heterozygosity (LOH) are just a few of the tumor suppressors that have been altered in colorectal cancer(14).The combined effects of cisplatin and Cassia fistula on the colon cancer cell line LS47T were assessed in the current investigation. These results showed that cisplatin and Cassia fistula together had a higher inhibitory effect on the colon cancer cell line than cisplatin and Cassia fistula separately. According to the data, cisplatin's IC₅₀ values for LS47T cells were 43.5 μ M.

CONCLUSION

Furthermore, MTT analysis was performed to investigate the synergistic effect of Cassia fistula and cisplatin on colon cancer cells. This suggested that Cassia fistula had a protective effect

against colon cancer cells cytotoxicity. The addition of cisplatin showed that the anticancer medication was more effective and had fewer negative effects when used together. Thus, this investigation showed that the Cassia fistula treatment in combination effectively reduced cell proliferation.

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