Synergistic Effects of *Cassia fistula* Extract Combination with Cisplatin on the Regulation of MicroRNA-145 and Gene Expression in Colon Cancer Cell Line SW480

Rana Talib Al-Muswie, Sabah H. Enayah[1], Rana A. Ghaleb[2]

Department of Basic Science, Dentistry College, Thi-Qar University, Thi-Qar, [1]Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, [2]Department of Human Anatomy, College of Medicine, University of Babylon, Hilla City, Iraq

Abstract

Background: Combination therapy is an effective strategy for inhibiting cancer cells and stimulating the apoptosis gene, so the potential synergistic effects of combining *Cassia fistula* extract with cisplatin chemotherapy used in clinical practice have been studied. **Objective:** The study aimed to investigate the anticancer effects of *C. fistula* extract *in vitro* as well as the precise molecular mechanisms. **Materials and Methods:** For 24 h, the human colon cancer cell line SW480 was given a variety of doses of cisplatin and plant extract, as well as cisplatin with various concentrations of *C. fistula*. MTT assay was used to assess the cytotoxicity of the cisplatin, plant extract, half maximal inhibitory concentration (IC_{50}) of cisplatin with plant, and combination of cisplatin and plant against colon cancer cells. The expression of microRNA, BAX, and BCL2 was analyzed using real-time polymerase chain reaction (qPCR). **Results:** The results showed that *C. fistula* extract exhibited higher cytotoxicity against colon cancer cells compared with untreated cells and showed synergistic effects when combined with cisplatin (CDDP). *Cassia fistula* extract induced cell apoptosis via the intrinsic (Bax and Bcl-2) apoptotic pathways and regulated the level expression of microRNA-145. In the SW480 cell line of colon cancer studies, *C. fistula* extract suppressed tumor growth through the inhibition of proliferation and induction of apoptosis. Our results showed synergistic effects when combined with IC_{50} of CDDP with Cassia extract through increasing the level of gene expression of Bax and reduced level expression of Bcl2. Also the regulated expression of microRNA-145. **Conclusion:** This study indicated that the synergistic of *C. fistula* extract and cisplatin significantly induce apoptosis by regulating the microRNA-145 gene, which is related to Bax and Bcl2 expression. Therefore, *C. fistula*, according to its cytotoxic and apoptotic activities, might be considered as a novel finding for the treatment of colon cancer.

Keywords: *Cassia fistula* extract, cisplatin gene expression and microRNA-145 expression, colon cancer

Introduction

Colon cancer is the second most common type of cancer in women (after breast cancer), whereas it is the third most prevalent type in men (after lung and prostate cancer).[1] Environmental factors, lifestyle factors, and dietary variables are the three biggest risk factors for colorectal cancer. These include habits such as smoking, drinking alcohol, not exercising enough, and not being active enough. They also include eating a diet heavy in fat and low in fiber. Many of these risk factors are under personal control and are preventable. The risk variables always include genetics, inheritance, and age.[2] The growth of colorectal cancer has been inhibited by compounds that are naturally present, and this has been the focus of research in recent years. One kind of molecule that has drawn a lot of interest recently is polyphenols, which are plant secondary metabolites and have been shown to improve human health and lower the risk of cancer.[3,4]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_33_23

Address for correspondence: Dr. Rana Talib Al-Musawie, Department of Basic of Science, College of Dentistry, University of Thi-Qar, Thi-Qar, 64001, Iraq.
E-mail: rana-almusawie@utq.edu.iq

Submission: 10-Jan-2023 **Accepted:** 24-Jan-2023 **Published:** 02-Feb-2024

How to cite this article: Al-Muswie RT, Enayah SH, Ghaleb RA. Synergistic effects of *Cassia fistula* extract combination with cisplatin on the regulation of microRNA-145 and gene expression in colon cancer cell line SW480. Med J Babylon 2023;20:670-80.

Extracts from the *Cassia fistula* plant have been put to use for a variety of pharmacological purposes, including anti-inflammatory treatment,[5] antibacterial,[6] wound healing,[7] and antioxidant properties (MCF-7 and SiHa cell lines for anticancer activity).[8] The volume of tumor and the number of live tumor cells were both decreased by the methanolic extract of *C. fistula* seed, as well as an extension of the mice's life span in Ehrlich ascites carcinoma (EAC tumor-bearing mice).[9,11] Chemotherapy can be administered either before or after surgery as an adjuvant treatment for solid tumors. Other patients who initially react to chemotherapy with remission may experience treatment resistance and relapse if cytostatics are repeatedly administered to them.[12,13] Endogenous noncoding RNAs known as microRNAs typically have a mature length of 22 nucleotides. MiRNAs induce gene silencing by destabilizing target mRNA or halting translation by complementary pairing with the 3′-untranslated region (3′-UTR) of the target mRNA.[14,15] According to reports, miRNAs play a key role in controlling how colon cancer progresses.[16] One such microRNA, identified as dysregulated in several of cancer forms, is microRNA (miR)-145. For instance, it has been noted that prostate and stomach cancer strongly downregulate miR145 expression.[17] Furthermore, miR-145's function in regulating cancer cell motility, invasion, and proliferation has been demonstrated. For instance, Sachdeva and Mo[18] discovered that miR-145 inhibits breast cancer cell's ability to proliferate and metastasize. The function of miR-145 and its therapeutic potential in colon cancer cells have not, however, been studied. In the current work, miR-145 expression in colon cancer cells was investigated. It was discovered that all colon cancer cell line SW480 dramatically downregulated the expression of miR-145. Additionally, miR-145 expression in colon cancer cells reduced the growth of these cells by causing them to undergo apoptosis by targeting Bax and Bcl2. The effects of miR-145 overexpression on apoptosis genes were also examined, and it was discovered that miR-145 reduced tumor growth. This study aimed to elucidate the medicinal plant *C. fistula*'s anticancer effects through analysis, understanding the cytotoxicity of the extracts and their combination with cisplatin on colon cancer cells, and further analysis of the pathway leading to apoptosis activation in these cells.

Material and Methods

Cassia fistula extract and cisplatin preparation

Collecting dry fruits and seeds of *C. Fistula* and then washing and drying the plant with distilled water. According to Bargah and Kushwaha[19] extractions consisted of aqueous extracts. by weight 20 g from the powder of the plant and then a put in pyrex beaker and 100 mL of boiling distilled water was added, then left for 30 min, then put the beaker on the heater overnight with a continuous magnetic stirrer, after that extract was centrifuged with 2500 rotary/min for 10 min, The extract obtained was put through a paper Whatman Filter and left overnight, after that we put the extract in the oven at 40–45. Weight extract and dissolved in a suitable amount of water in order to obtain the final stock concentration (1000 µg/mL) and sterile by using a Millipore filter of 0.22 m. The 50-mL vials of cisplatin, 1 mg/mL, were bought from the French company Mylan.

Cell line, growth conditions, and treatment

The SW480 cell line was purchased from the National Cell Bank of Iran affiliated to the Pasteur Institute of Tehran, Iran. RPMI1640 medium (Biowest) and 10% fetal bovine serum were used for the culture (FBS; Biosera, South America). For cell passaging, 1% Tripsin EDTA (ATOCEL Company, Budapest) was applied and incubated once the cells had reached 80% confluence. Then, 10% FBS + 1% PS was added to make up a full cell culture medium. Penicillin and streptomycin were added to a solution to suppress the growth of a range of Gram-positive and Gram-negative bacteria.

In vitro cytotoxicity assay

Cell proliferation was assessed after 24 h using the 3 (4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay. The resultant solution of MTT powder in phosphate buffered saline medium was added to the wells of a 96-well microplate containing a cell line that had been divided into five groups. The first group of cel*l*s no treated as control. The Second group of cells treated with different concentrations of drug cisplatin(15.60, 31.25 62.5, 125, 250, and 500) µg/mL; The Third group treated at different concentration of *C. fistula* extract (15.60, 31.25, 62.5, 125, 250, and 500 µg/mL), The Fourth group combinations of the IC_{50} CDDP + *C. fistula* and, the fifth group treated with combination of equal concentration of CDDP (15.60, 31.25, 62.5, 125, 250, and 500) µg/mL + *C. fistula* extract (15.60, 31.25, 62.5, 125, 250, and 500) µg/mL. Dimethyl sulfoxide was added to the wells after 4 h of incubation at 37°C, and the absorption rate was measured at 570 nm.

Real-time polymerase chain reaction evaluation of gene expression

Expression levels of Bax and Bcl2 were assessed using real-time polymerase chain reaction (PCR) with the relevant primers [Table 1]. Real-time PCR was performed to analyze the gene expression during cell death due to treatment of the cancer cells with the combination of extract and chemotherapy. Cells were harvested after treatment with different concentrations (15.60, 31.25, 62.5, 125, 250, and 500 µg/mL) of the combination. The relative gene expression of Bax and Bcl2 and normalized by actin gene were estimated by rt real-time PCR (Vottor

Table 1: Sequence of genes primers for qRT-PCR

Primers	Primers sequencing	References
Bax(F)	5′-GGTTGTCGCCCTTTTCTA-3′	Eimani *et al.* (2014)[20]
Bax(R	5′-CGGAGGAAGTCCAATGTC-3′	
Bcl2 (F)	5′-GATGTGATGCCTCTGCGAAG-3′	Eimani *et al.* (2014)[20]
Bcl2 (R)	5′-CATGCTGATGTCTCTGGAATCT-3′	
Housekeeping	Primer sequencing	
Actin		
(F)	GCGAGAAGATGACCCAGAT	Eimani *et al.* (2014)[20]
(R)	GAGGCGTACAGGGATAGC	

Table 2: qPCR thermo cycler conditions

No.	Step	Temperature	Duration	Cycle
1	Reverse transcription	45°C	10 min	Hold step
2	Denaturation	94°C	30 s	
3	Denaturation	95°C	10 s	40 cycle
4	Annealing	60°C	30 s	
5	Acquired on green	95°C	5 s	

Table 3: PCR primer pairs for micRNA-145

Primer ID	Sequence (5′→3′)	Length (bp)	GC (%)	GC (%)
S145f	GGACGGTAGCAAGCAAAGAGTGTGAGGGATTCCTG	35	54	71.9
S145r	GGGATTCTGGAAGATGATGATGACGTCCAGTTTTC	35	46	67.6
U145f	GGACGGTAGCAAGCAAAGAGTGTG	24	54	64.4
U145r	GGGATTCTGGAAGATGATGATGAC	24	46	60.5
UUU6f	CTCGCTTCGGCAGCACA3	17	65	62.9
UUU6r	AACGCTTCACGAATTTGCGT	20	45	58.8

Table 4: Recommended QPCR reaction components and conditions (20 μL reaction system)

Component	Volume	Final concentration
RNA template	1 pg–100 ng	As required
Forward primer (10 μM)	0.5 μL	0.2 μL
Reverse primer	0.5 μL	0.2 μL
2× perfectStarTMGreen one –step qPCR SuperMix	10 μL	1×
TransScriptRGreen one-step RT/R1 Enzyme Mix	0.4 μL	–
RNase-free water	Variable	–
Total volume	20 μL	–

gene Q, Qia gene Germ) employing in the primer in table. ONE STEP Syper green master mix used amplified target gene, the reaction mixture, and throcycle condition, as shown in Table 2.

After amplification, the specificity and salacity were evaluated by melting curve analyzed by roter gene Q software (Q gene Germany), and ΔΔCD method was employed according to livac (ΔΔCD).

Evaluation of micRNA-145 using real-time PCR

Total RNA in colon cancer cells was isolated using GENEzol reagent (TriRNA Pure kit) according to the manufacturer's protocol. Complementary DNA (cDNA) for mRNA and miRNA were synthesized using the TransScript Green One-Step qRT-PCR SuperMix according to the manufacturer's instructions. The relative level of miR-145 was determined by qRT-PCR using gene specific primers [Table 3]. U6 was validated as the normalizer, since which expression showed minimal variation in different cell line. The RT primers were created in the following manner: miR-145 [Table 4]:

Statistical analysis

The results of each analysis were carried in triplicate and are shown as means standard deviation (SD). The

Statistical Package for the Social Sciences (SPSS) software program, version 25.0 was used to conduct the statistical analysis using Student's *t* tests. A value of $P < 0.05$ was considered statistically significant.

Ethical approval

A local ethics committee examined and approved the study protocol, subject information, and consent form in accordance with 3/11/903, dated June 2, 2021.

Results

The MTT assay is based on the ability to interact with living cells, which results in the reduction of a yellow tetrazolium dye to a purple formazan product. The objective of the present study was to determine the effect of cisplatin (CDDP) and *C. fistula* extract on cell proliferation and growth using the MTT assay. Results showed that 500 µg extract depicted the least cell viability compared to another concentration. Hence, it was most toxic to the cancer cells.

Cytotoxic effects of cisplatin on colon cancer cell line SW480

MTT assay was used to determine the cytotoxic effects of cisplatin on colon cancer cell line. The results of the analysis of all the cells exposed to cisplatin at several concentrations (0–500 g/mL concentrations) for 24 hours are depicted in Figure 1. The vitality of the cells decreased with increasing cisplatin concentration, as seen in Figure 1 in the cell line. In a concentration-dependent function, the survival percentage of drug-treated cells was significantly lower than the untreated cells ($P < 0.05$). At high doses (500 g/mL), cisplatin exhibited a growth-inhibiting effect on colon cancer. The drug dosage against the colon cancer cell line at 500 g/mL caused the highest reduction in the percentage of cell viability. The assay showed the drug effect on cell line SW480 in all concentrations compared with untreated cells. In evident increased in toxicity was observed at a concentration of 500 µM starting from 15. 62 µg/mL, respectively.

Cytotoxic effects of *C. fistula* extract on SW480 colon cancer cells

Figure 2 shows that the cell viability percent of SW480 colonic cancer cell line by extract plant *C. fistula* treatment after 24 h of incubation were significant ($P < 0.05$) as compared with control group (cancer cells without treatment) in the concentrations (15.60, 31.25, 62.5, 250, and 500 µg/mL). After treatment with *C. fistula* extract at various concentrations, inhibitory concentrations were observed. As the plant extract's concentration increased, a decline in cell viability was seen. The survival rate of colon cancer cells was significantly lower after treatment with *C. fistula* extract than it was for the untreated cell ($P < 0.05$). The colon cancer cell line was exposed to a plant extract at a concentration of 500 g/mL, which resulted in the highest decrease in the cell viability percentage.

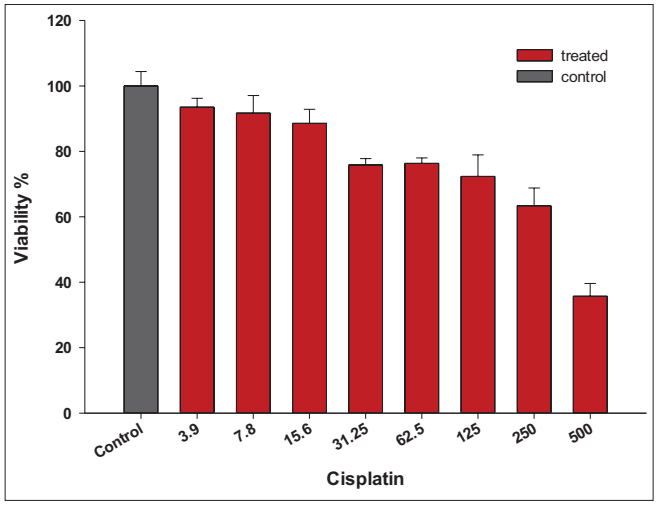

Figure 1: Cell viability of cisplatin in SW480 cell line assessed by MTT reagent protocol. Cells were treated with different concentrations of cisplatin (0, 15.6, 31.25, 62.5, 125, 250, and 500 μM) for 24 h

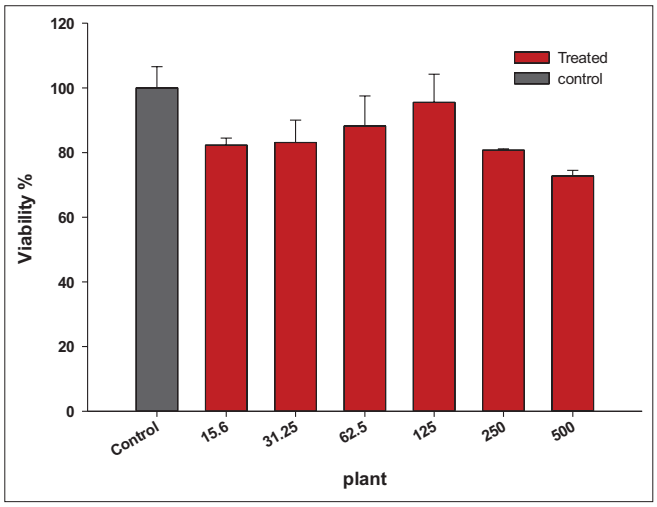

Figure 2: Effect of *Cassia fistula* on the viability of SW480 cells. Cells were treated with extract at concentrations of (15.60, 31.25, 62.5, 125, 250, and 500 μg/mL) and measured after 24 hours. The viability of cells was determined with the MTT assay

Cytotoxic effects of combination IC_{50} from cisplatin (CDDP) with *Cassia fistula*

The cytotoxic effects of the combination on colon cancer were assessed using the MTT test. Data were evaluated after cells were exposed to combinations of IC50 of cisplatin and *C. fistula* at varying doses (0–500 g/mL concentrations) for 24 h [Figure 3]. Additionally, the inhibitory concentrations following the administration of IC_{50} of cisplatin at various concentrations to an extract of *C. fistula* were displayed. A decrease in cell viability was seen as the combination's concentration was increased. After treatment with combination, the survival percentage

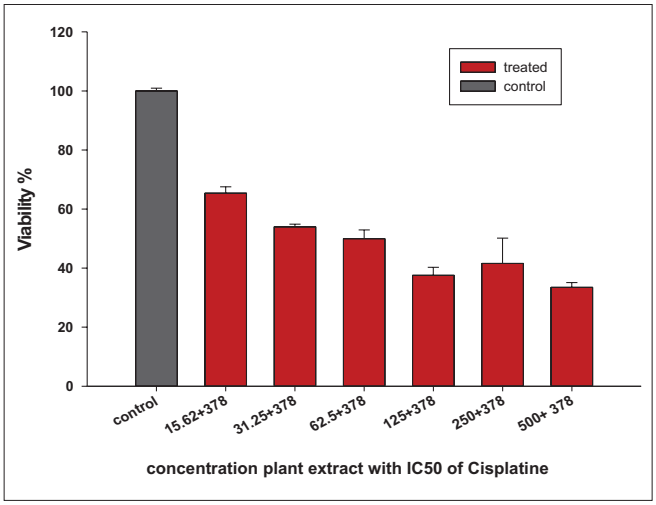

Figure 3: Effect of combination of IC50 cisplatin and *Cassia fistula* on the viability of SW480 cells. Cells were treated with IC_{50} of drug and extract concentrations of (15.60, 31.25, 62.5, 125, 250, and 500 μg/mL) and measured after 24 hours. The viability of cells was determined with the MTT assay

of colon cancer cells was considerably decreased than the untreated cell ($P < 0.05$). The combination had a growth inhibitory effect on the colon cancer cell line SW480 in high concentrations (500 μg/mL). When combined with cisplatin at a concentration of 500 g/mL, plant extract significantly decreased the percentage of cells that were viable.

Cytotoxic effects of CDDP in combination with *C. fistula*

The cell viability percent of SW480 colonic cancer cell line by a combination of equal concentration of Cisplatin and *C. fistula*, treatment after 24 hours incubation were significant ($P < 0.05$) as compared to control group (cancer cells without treatment) in all concentrations (15.60, 31.25, 62.5, 125, 250 and 500 μg/mL). Furthermore, the inhibitory concentrations after treatment of *C. fistula* extract with cisplatin at different concentrations were presented in Figure 4. A decrease in cell viability was seen as the combination's concentration was increased. After receiving combination therapy, colon cancer cells had a significantly lower survival rate than untreated cells ($P < 0.05$) in concentration-dependent function. In high doses (500 g/mL), the mixture showed a growth-inhibitory effect on the colon cancer cell line SW480. When combined with cisplatin at a dosage of 500 g/mL, plant extract significantly decreased the proportion of cells that were viable.

Gene Expression Analysis

Effects of cisplatin (CDDP) on the expression levels of Bax and Bcl2

The gene expression level changes of genes in response to treatment with cisplatin was assessed by methodologies real-time PCR.

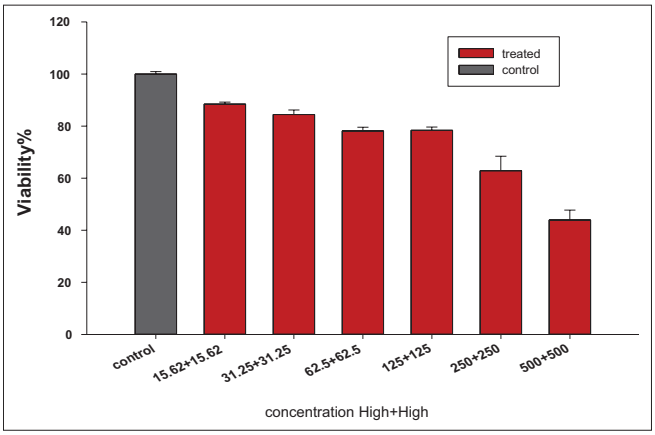

Figure 4: Effect of combination of cisplatin and *Cassia fistula* on the viability of SW480 cells. Cells were treated with high concentration of drug and high concentration of extract (15.60 + 15.60, 31.25 + 31.25, 62.5 + 62.5, 125 + 125, 250 + 250 and 500 + 500 μg/mL) and measured after 24 hours. The viability of cells was determined with the MTT assay

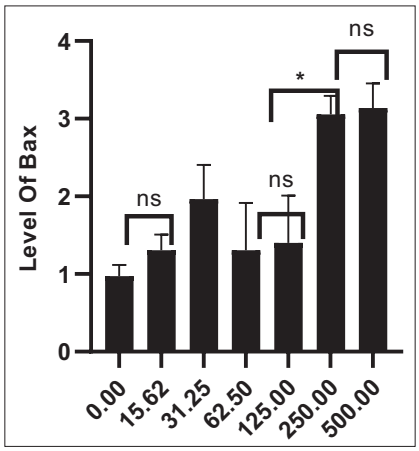

Figure 5: Changes in mRNA levels of Bax gene of colon cancer cell lines in response to treatment with cisplatin comparison with control. *P-value < 0.05; ns = non significant

Results indicated that treatment of colon cancer cell line SW480 with drug (CDDP) leads to upregulation of Bax gene in cell line compared with untreated cell. Figure 5 showed expression level increased in concentration (250 and 500 μg/mL) compared with other concentrations. Regards to confirmation of the changes that occurred in gene expression levels in response to treatments. It was observed that the concentration (31.25, 250, and 500) μg/mL of CDDP showed a significant fold decrease in the Bcl2 expression compared with untreated cells. Results indicated that, treatment of colon cancer cell line SW480 with drug CDDP leads to downregulation of Bcl2 gene [Figure 6].

Effects of *C. fistula* on the expression levels of bax and Bcl2

To identify the apoptotic pathway activated by treatment with *C. fistula* extract in colon cancer cell line SW480, we

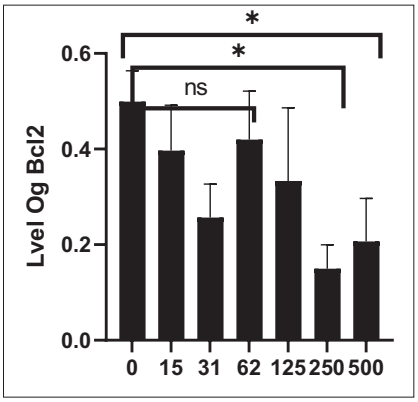

Figure 6: Changes in mRNA levels of Bcl2 gene of colon cancer cell lines in response to treatment with cisplatin comparison with control. *P-value < 0.05; ns = non significant

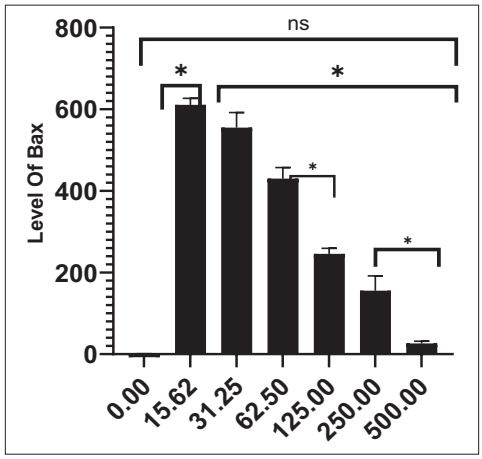

Figure 7: Changes in mRNA levels of Bax gene of colon cancer cell lines in response to treatment with *Cassia fistula* comparison with control. *P-value < 0.05; ns = non significant

found that C.F. extract activated intrinsic Bax, apoptotic pathways [Figure 7]. Furthermore, expression level increased in concentration (15.62 and 31.25 and 62.5 µg/mL) compared with untreated cell but no significant between concentration of (500 µg/mL) with control group. The level of Bcl2 gene in cells treated with concentration (31.25, 62.5, and 250 µg/mL) of *C. fistula* was increased in level of gene expression [Figure 8], but no significant in cells treated with extract in concentration 15.62 and125 µg/mL) compared with untreated cell. Also expression level decreased in concentration of 500 µg/mL compared with untreated cells.

Effects of IC_{50} of cisplatin in combination with *C. fistula* on the expression levels of bax and Bcl2

Results of real-time PCR indicated that treatment of SW480 cell line with IC_{50} of CDDP + C. F. extract leads to upregulation of the Bax gene [Figure 9]. The expression level of the Bax gene in both plant extract with the drug utilized cell lines was upregulated significantly and

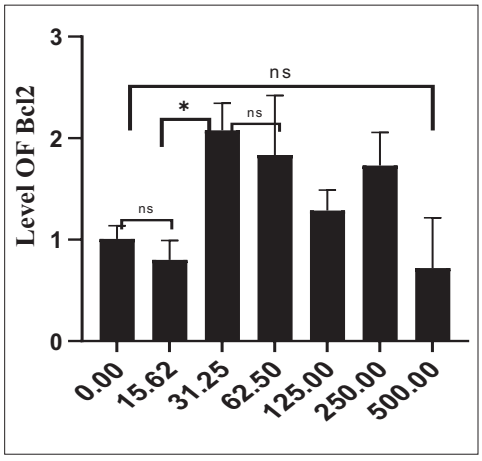

Figure 8: Changes in mRNA levels of Bcl2 gene of colon cancer cell lines in response to treatment with *Cassia fistula* comparison with control. *P-value < 0.05; ns = non significant

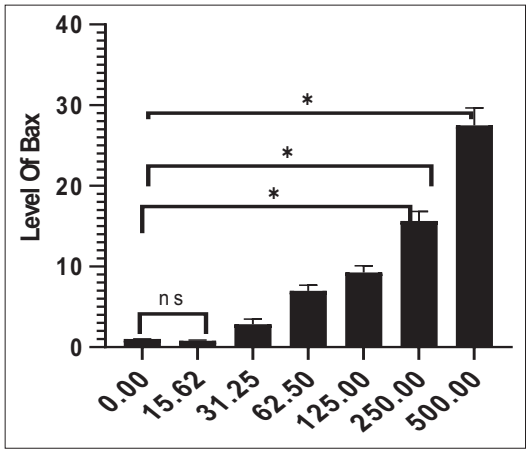

Figure 9: Changes in mRNA levels of bax gene of colon cancer cell lines in response to treatment combination of cisplatin LC50 with *Cassia fistula* comparison with control

increased in the concentration of 500 µg/mL compared with other concentrations and untreated cells, but did not have significant toxicity on cancer cells after treated with 15.62 µg/mL concentration of combined of IC_{50} of CDDP and *C. fistula*. The gene expression level change of the Bcl2 gene in response to treatment with IC_{50} of CDDP and C.F. extract, and the treatment leads to downregulation of the Bcl2 gene [Figure 10]. The expression level of the Bcl2 gene in both plant extract with the drug utilized cell lines was downregulated significantly and decreased in all concentrations compared with untreated cells.

Effects of cisplatin in combination with *C. fistula* on the expression levels of bax and Bcl2

Combined equal concentrations of the drug cisplatin and *C. fistula* extract were also tested on cell line. Results of cell counting after 24, are shown in Figure 11. It is noteworthy that 250 µg/mL of combination increased expression level

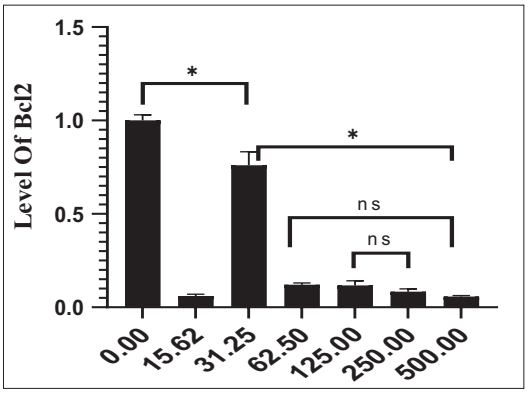

Figure 10: Changes in mRNA levels of Bcl2 gene of colon cancer cell lines in response to treatment combination of cisplatin LC_{50} with *Cassia fistula* comparison with control

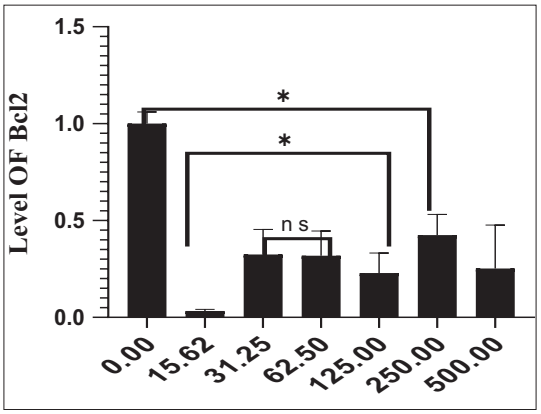

Figure 12: Changes in mRNA levels of Bcl2 gene of colon cancer cell lines in response to treatment combination of cisplatin with *Cassia fistula* comparison with control

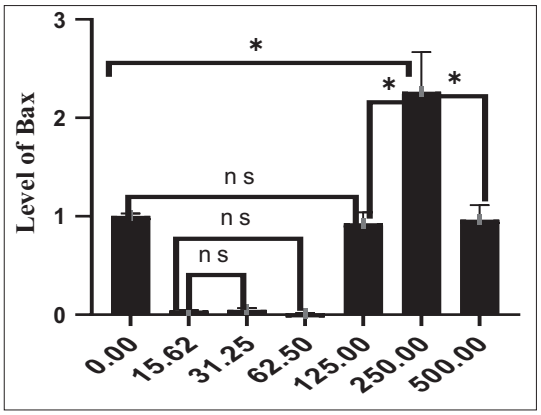

Figure 11: Changes in mRNA levels of Bax gene of colon cancer cell lines in response to treatment combination of cisplatin with *Cassia fistula* comparison with control

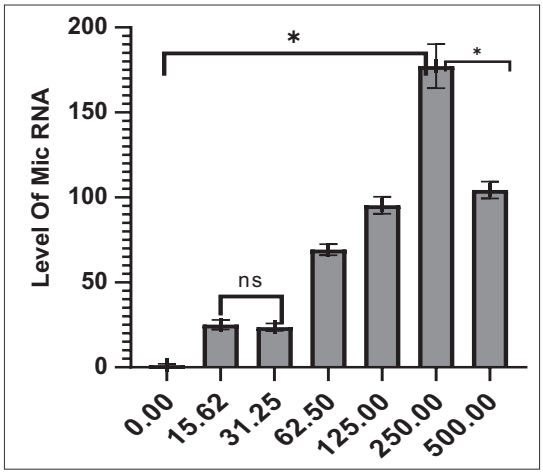

Figure 13: Changes in mRNA levels of MicRNA gene of colon cancer cell lines in response to treatment of cisplatin comparison with control. **P*-value < 0.05; ns = nonsignificant **P*-value < 0.05; ns = nonsignificant

of Bax compared with other concentrations and untreated cells but did not have significant toxicity on cancer cells after treated with (125 and 500) µg/mL concentration of combined of drug an extract with equal concentration.

Expression level of Bcl2 gene in group combination of cisplatin with plant extract cell lines was downregulated significantly in all concentrations compared with untreated cells. Cisplatin and *C. fistula* extract treatment significantly reduced the expression of the Bcl-2 gene, and this decrease was seen in all concentrations when compared to untreated cells. In comparison to other concentrations and untreated cells, the expression level of the Bcl2 gene was significantly downregulated in both plant extract- and drug-treated cell line at concentrations of 15.62, 125, and 500 g/mL [Figure 12].

miR-145 regulated the expression of bax and Bcl2

SW480 with drug cisplatin leads to upregulation of MicroRNA gene in cell line compared with untreated cells. Figure 13 shows expression level increased in all concentration compared with untreated cells. Also expression level increased in concentration of (250 µg/mL) compared with other concentrations and untreated cells. Expression of miR-145 was activated by treatment with *C. fistula* extract in colon cancer cell line SW480, it was found that C.F. extract activated miR-145 [Figure 14]. Furthermore, expression level increased in concentration (500 µg/mL) compared with untreated cells and other concentrations. Also there is no significant difference between all concentrations compared with untreated cells.

Combined concentrations of the drug IC_{50} from Cisplatin with *C. fistula* extract were also tested on cell line. Results from cell counting after 24 are represented in Figure 15. It is noteworthy that all concentration increased the expression level of microRNA compared with untreated cells. Also, expression level increased in concentration (500 µg/mL) compared with untreated cells and other concentrations. The gene expression level change of miR-145 gene in response to treatment with equal concentration of CDDP and C.F. extract were assessed real-time PCR indicated

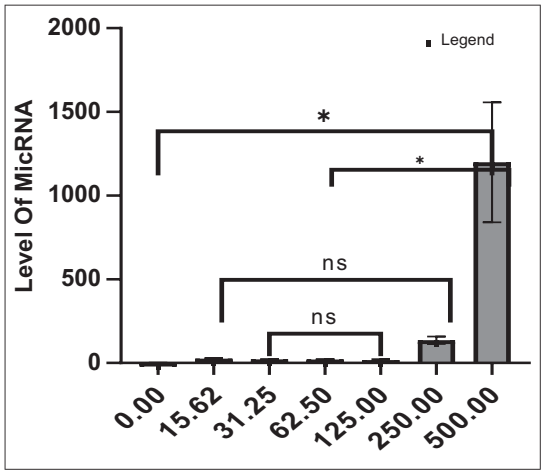

Figure 14: Changes in mRNA levels of MicRNA gene of colon cancer cell lines in response to treatment with *Cassia fistula* comparison with control. *P-value < 0.05; ns = nonsignificant

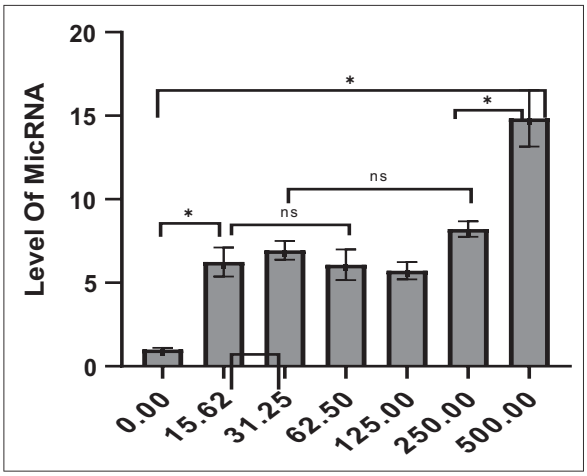

Figure 15: Changes in mRNA levels of MicRNA gene of colon cancer cell lines in response to treatment combination of cisplatin IC50 with *Cassia fistula* comparison with control. *P-value < 0.05; ns = nonsignificant

that, treatment of SW480 cell line with combination of Cisplatin + *C. fistula* extract leads to downregulation of miR-145 gene [Figure 16]. The correlation between micRNA and Apoptosis genes of Bax and Bcl2. Real-time PCR results revealed that the pro-apoptosis genes (in the cells were exposed to cisplatin, Bax was upregulated while Bcl-2, an anti-apoptotic gene, was downregulated, *C. fistula*, IC_{50} from Cisplatin + extract and combination with equal concentration of chemotherapy and extract of plant. The correlation between miR-145 and the level of gene expression for Bax and Bcl2, when miR-145 expression increases in the colon cancer cell line promotes SW480 cell apoptosis. The results demonstrated that miR-145 shows a significant increase in SW480 cell apoptosis gene Bax, but, the expression of Bcl2 was significantly decreased in these groups, cisplatin, *C. fistula* and treated with IC_{50} from Cisplatin. Also, synergistic cytotoxic effects

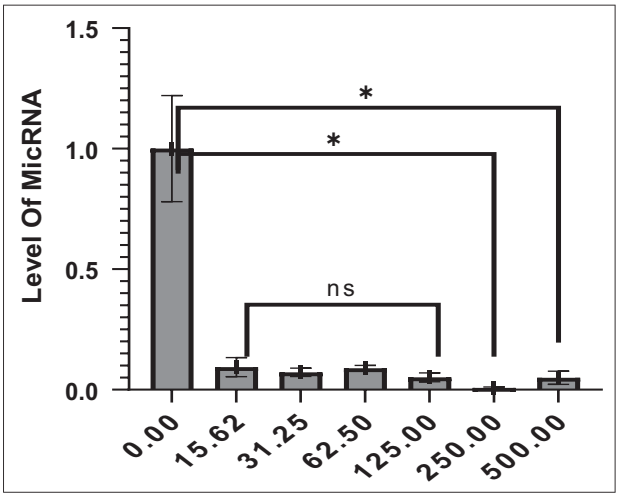

Figure 16: Changes in mRNA levels of MicRNA gene of colon cancer cell lines in response to treatment combination of cisplatin with *Cassia fistula* comparison with control. * P-value < 0.05; ns = nonsignificant

of IC_{50} Cisplatin with *C. fistula* on the expression levels of micRNA-145 gene.

Discussion

Due to its ability to provide therapeutic effects that are additive, potentiating, or synergistic and overcome drug resistance, combination chemotherapy has proven to be more effective than single-drug therapy in the treatment of cancer.[21,22] A single drug can promote the development of chemoresistance mutations and tumor relapse by activating alternate molecular and cellular pathways in tumor cells. Co-administration of two or more medications, however, boosts tumor cell killing while decreasing the chance that mutations would lead to the emergence of cancer treatment resistance.[23,24] One of the most famous anticancer medications is cisplatin, which is used to treat ovarian, prostate, head and neck, lung, bladder, and other cancers. Apoptosis is carried forward by CDDP's interaction with DNA, which results in the formation of DNA adducts that obstruct DNA synthesis and repair.[25] Cisplatin is commonly used with other medications to improve therapeutic efficacy and reduce side effects. The purpose of this investigation was to determine the cytotoxic effect of the combination *C. fistula* extract with cisplatin chemotherapy in colon cancer cell line SW480, one of the most prevalent types of cancer worldwide, and to identify the likely pathways of action by correlating microRNA with the apoptosis genes Bax and Bcl2.

In vitro tests are carried out first to ascertain any compound's possible biological advantages. *In vitro* colon cancer models for cancer research often use the human colon carcinoma-derived SW480 cell line. Therefore, an in vitro experiment using the colon cancer cell line SW480 was devised. A substance used to treat cancer must meet

a variety of criteria in order to be effective, including not harming healthy cells. Cisplatin is often used in combination with extract of plants, with positive results. The goal is to produce synergistic or at least additive effects in killing cancer cells, while reducing side effects.

In the current research, cytotoxic potential of combination extract and chemotherapy was assessed by MTT assay against cancer cell lines (SW480). Assay is based on the reduction of yellow tetrazolium salt (MTT) to a dark blue formazan by metabolically active cells.[26,27] The cells treated with *C. fistula*, IC_{50} of cisplatin, and combination showed a significant cytotoxic effect against cell line with a concentration of 500 µM, and no significant cytotoxicity to the untreated cell line was observed [Figures 1–4]. Plant products containing phenolic compounds exert antiproliferative effects on cancer cells by suppressing ROS-scavenging systems, deactivating pro-survival signals, activating apoptosis-related signals, producing DNA damage, and disrupting signaling pathways supportive of cancer cell proliferation.[28] Cell cycle analysis and flow cytometry were utilized to analyze apoptotic events and determine the mechanism underlying these plant extracts' cytotoxic effects on colon and prostate cancer cell lines.[29,30]

The results indicated that CDDP, *C. fistula*, IC_{50} of CDDP + *C. fistula* extract, and the study's chemotherapy and plant extract combination have the potential to induce apoptosis, such as delayed apoptosis, at various concentrations. Similar to this, numerous natural substances have anticancer or anticancer effects by promoting apoptotic pathways in cells that have undergone transformation throughout the carcinogenesis process.[31,32]

Bax and Bcl-2, two mitochondrial apoptosis-related genes, had their transcription and translation levels of expression examined in this study. Upregulation of Bax and downregulation of Bcl-2 are seen in a gene expression analysis [Figures 7 and 8].

These results suggest that pathways associated with the mitochondria-based pathway are used by *C. fistula* bioactive to cause apoptosis in SW480 cells. This is in agreement with the results that were reported.[33,34] In this study, levels of gene expression for mitochondrial apoptosis (Bax and Bcl2) were examined. A gene expression analysis reveals a higher level of Bax. This study's results are consistent with the results of a previous study, which found that bioactive plant components from *Setarla parviflora* and *Ducrosia flabellifolia* cause apoptosis in HCT-116 and PC3 cells through mechanisms connected to the mitochondrial pathway.[33] According to the results of a different study, the methanol extract of *C. fistula* prevents prostate cancer cells from proliferating by triggering apoptosis. According to the results of this research, the methanol extract stops prostate cell division and causes cell arrest.[35] Because of how they affect cell division, apoptosis, and invasion, improperly expressed miRNAs have been associated with cancer in a number of studies published in recent years Thus, miRNAs may be used as biomarkers for the detection and prediction of various cancers, including CRC. This study found that as compared to control cells, the expression of miR-145 was dramatically downregulated in CRC cell lines. Additionally, treatment with cisplatin, *C. fistula*, and combined IC_{50} of cisplatin + *C. fistula* resulted in an increase in miR-145 expression. To the best of our knowledge, in order to clarify the process involved in the genesis and progression of cancer, the identification of specific miRNA target genes is required. The findings of this study are consistent with earlier studies that showed miRNAs regulate their target genes by binding to the 3′-UTR of mRNA. Furthermore, micRNA increased in cells treated with cisplatin, *C. fistula*, and combination IC_{50} of cisplatin + *C. fistula* extract. The most widely prescribed medication for the chemotherapy treatment of CRC and other cancers, including ovarian cancer, is cisplatin[36] lung.[37] The increased expression of genes associated to drug resistance, the decreased accumulation of medicines, the increased levels of metallothionein in cells, and the improved DNA repair capacity are all effects of tumor cells' resistance.[38]

The results showed that cells treated with cisplatin or in combination with cisplatin proliferate less frequently and undergo more apoptosis. Additionally, in combination-treated cells, the antiapoptotic gene Bcl-2 was downregulated while the proapoptosis gene Bax was increased. Apoptosis can be predicted by the ratio of Bax and Bcl-2.[39] MiR-145 played an antitumor role in CRC in the current investigation. The proapoptotic genes Bax was elevated and the antiapoptotic gene Bcl-2 was downregulated in cells treated with IC_{50} of cisplatin + *C. fistula* extract.

In the current investigation, it was found that primary colon adenocarcinoma cells drastically downregulated miR-145 expression. Additionally, miR-145 expression induced apoptosis in colon cancer cells, which reduced their ability to proliferate. Additionally, the migration and invasion of SW480 colon adenocarcinoma cells were reduced by the overexpression of miR-145. For instance, miR-145 has been found to inhibit the main colon's growth and metastasis.[40] Similar to this, Yin *et al.* showed that miR-145 targeted octamer-binding transcription factor 4 and prevented the growth of lung cancer.[41] Additionally, research on miRNA expression has identified the function of miR-145 in the metastasis of colorectal cancer.[42] The findings of the current investigation indicate that miR145 has cancer-specific effects. A detailed analysis at the underlying mechanism showed that miR-145 targeted the apoptosis genes Bax and Bcl2 and had a silencing impact on the growth of primary colon cancer cells as a result of miR-145 overexpression.

The most frequent effects of in vitro phytochemical modification on cell growth and proliferation, metastasis, and apoptosis pathways were observed. The use of phytochemicals in established chemotherapy regimens is possible. Curcumin in combination, for instance, may be an acceptable treatment if an abnormal dysregulation of the Wnt pathway was observed in a particular colon tumor, as it was discovered to downregulate the Wnt pathway in SW480 cells.[43]

Conclusion

The results of this study indicated that *C. fistula* has a therapeutic effect against colon cancer alone and when combined with cisplatin, as it was found to have a synergistic effect through the toxic effect on cancer cells, which can be a promising alternative to resisting colon cancer. Treatments with each plant's extract, cisplatin's IC50 with the extract, and combinations of cisplatin and extract at equal concentrations all had an effect on the level of gene expression. The Bax gene expression was upregulated while Bcl-2 gene expression was downregulated, and there was a correlation between increased microRNA and genes Bax and Bcl2. In this study, gene expression was correlated with elevated microRNA-145 levels. The extracts contained antiproliferative bioactive compounds, according to the results, which call for larger, more thorough investigations that concentrate on fractionating and characterizing these bioactive compounds as well as researching their various levels of action.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Vargas AJ, Thompson PA. Diet and nutrient factors in colorectal cancer risk. Nutr Clin Pract 2012;27:613-23.
2. Haggar FA, Boushey RP. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. Clin Colon Rectal Surgery 2009;22:191-7.
3. Zeriouh W. Phenolic extract from oleaster (*Olea europaea* var. Sylvestris) leaves reduces colon cancer growth and induces caspase-dependent apoptosis in colon cancer cells via the mitochondrial apoptotic pathway. PLoS One 2017;12:e0176574.
4. Tresserra-Rimbau A, Lamuela-Raventos RM, Moreno JJ. Polyphenols, food and pharma: Current knowledge and directions for future research. Biochem Pharmacol 2018;156:186-95.
5. Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, *et al*. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J Biol Chem 2005;280:31182-9.
6. Irshad M, Aijaz A, Zafaryab M, Ahmad F, Manzoor N, Singh M, *et al*. Composition of *Cassia fistula* oil and its antifungal activity by disrupting ergosterol biosynthesis. Nat Prod Commun 2013;8:261-4.
7. Irshad M, Zafaryab M, Singh M, Rizvi MMA. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. Int J Med Chem 2012;2012:157125.
8. Nirmala A, Eliza J, Rajalakshm M, Priya E, Daisy P. Effect of hexane extract of *Cassia fistula* barks on blood glucose and lipid profile in streptozotocin diabetic rats. Int J Pharmacol 2008;4: 292-296.
9. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. J Ethnopharmacol 2000;72:151-6.
10. Green S, Konig DH. Treatment of acute myeloid leukemia in the era of genomics—achievements and persisting challenges. Front Genet 2020;11:480.
11. O'Donnell MR, Tallman MS, Abboud CN, Altman JK, Appelbaum FR, Arber DA, *et al*. Acute myeloid leukemia, version 3.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2017;15:926-57.
12. Mueller BU, Seipel K, Bacher U, Pabst T. Autologous transplantation for older adults with AML. Cancers 2018;10:340.
13. Zheng DW, Dong X, Pan P, Chen KW, Fan JX, Cheng SX, *et al*. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. Nat Biomed Eng 2019;3:717-28.
14. Cui Y, Li Y, Wang Z, Lei Q, Koizumi Y, Chiba A. Regulating twin boundary mobility by annealing in magnesium and its alloys. Int J Plast 2017;99:1-18.
15. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: Biomarkers, functions and therapy. Trends Mol Med 2014;20:460-9.
16. To KK, Tong CW, Wu M, Cho WC. MicroRNAs in the prognosis and therapy of colorectal cancer: From bench to bedside. World J Gastroenterol 2018;24:2949.
17. Ji W, Sun B, Su C. Targeting microRNAs in cancer gene therapy. Genes 2017;8:21.
18. Sachdeva M, Mo YY. miR-145-mediated suppression of cell growth, invasion and metastasis. Am J Transl Res 2010;2:170.
19. Bargah RK, Kushwaha PK. Extractions, phytochemical screening and in-vitro antioxidant activity of *Cassia fistula* extracts. Int J Res Pharm Chem 2017;7:518-24.
20. Eimani BG, Sanati MH, Houshmand M, Ataei M, Akbarian F, Shakhssalim N. Expression and prognostic significance of bcl-2 and bax in the progression and clinical outcome of transitional bladder cell carcinoma. Cell J (Yakhteh) 2014;15:356.
21. Jia J, Zhu F, Ma X, Cao ZW, Li YX, Chen YZ. Mechanisms of drug combinations: Interaction and network perspectives. Nat Rev Drug Discov 2009;8:111-28.
22. Hu Q, Sun W, Wang C, Gu Z. Recent advances of cocktail chemotherapy by combination drug delivery systems. Adv Drug Deliv Rev 2016;98:19-34.
23. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006;5:219-34.
24. Dear RF, McGeechan K, Jenkins MC, Barratt A, Tattersall MH, Wilcken N. Combination versus sequential single agent chemotherapy for metastatic breast cancer. Cochrane Database of Syst Rev 2013;12:CD008792.
25. Dasari, SP.B. Tchounwou. Cisplatin in cancer therapy: Molecular mechanisms of action. Eur J Pharmacol 2014;740:364-78.
26. Mosdam T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assay. J Immunol Methods 1983;65:55-63.
27. Chabuck ZAG, Al-Shukri MS, Abdul-Lateef LA. Effect of different purified Pseudomonas exopigments on vero-cell line and some pathogenic bacterial isolates. Med J Babylon 2017;14.
28. Chikara S, Nagaprashantha LD, Singhal J, Horne D, Awasthi S, Singhal SS. Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer Lett 2018;413:122-34.
29. Auyeung B, Taylor K, Hackett G, Baron-Cohen S. Foetal testosterone and autistic traits in 18 to 24-month-old children. Mol Autism 2010;1:11111-8.
30. Anwar A, Uddin N, Siddiqui BS, Siddiqui RA, Begum S, Choudhary MI. A natural flavonoid laws on aringenin induces cell

cycle arrest and apoptosis in HT-29 colorectal cancer cells by targeting multiple signalling pathways. Mol Biol Rep 2018;45:1339-48.
31. Bishayee AA, Darvesh S. Pomegranate-derived constituents as inducers of cell death: Implications in cancer prevention and therapy. Natural Compounds as Inducers of Cell Death 2012;1:33-47.
32. Mukhtar E, Mustafa Adhami V, Khan N, Mukhtar H. Apoptosis and autophagy induction as mechanism of cancer prevention by naturally occurring dietary agents. Curr Drug Targets 2012;13:1831-41.
33. Bhatia D, Mandal A, Nevo E, Bishayee A. Apoptosis-inducing effects of extracts from desert plants in HepG2 human hepatocarcinoma cells. Asian Pac J Trop Biomed 2015;5:87-92.
34. Ghaleb RA. The effect of diode laser 635 nm on mitochondrial membrane potential and apoptosis induction of CHO47 cells line. Med J Babylon 2016;13:7-16.
35. Irshad M, Mehdi SJ, Al-Fatlawi AA, Zafaryah Md, Ali A, Ahmad I, *et al*. Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines. J Biol Active Prod Nature 2014;4:158-70.
36. Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, *et al*. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. Theranostics 2018;8:3932.
37. Zhang J, Gu Y, Chen B. Mechanisms of drug resistance in acute myeloid leukemia. OncoTargets Ther 2019;12:1937.
38. Baguley BC. Multiple drug resistance mechanisms in cancer. Mol Biotechnol 2010;46:308-16.
39. Sun L, Li D, Song K, Wei J, Yao S, Li Z, *et al*. Exosomes derived from human umbilical cord mesenchymal stem cells protect against cisplatin-induced ovarian granulosa cell stress and apoptosis in vitro. Sci Rep 2017;7:1-13.
40. Gao S, Wang P, Hua Y, Xi H, Meng Z, Liu T, *et al*. ROR functions as a ceRNA to regulate Nanog expression by sponging miR-145 and predicts poor prognosis in pancreatic cancer. Oncotarget 2016;7:1608.
41. Yin R, Zhang S, Wu Y, Fan X, Jiang F, Zhang Z, *et al*. microRNA-145 suppresses lung adenocarcinoma-initiating cell proliferation by targeting OCT4. Oncol Rep 2011;25:1747-54.
42. Arndt GM, Dossey L, Cullen LM, Lai A, Druker R, Eisbacher M, *et al*. Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. BMC Cancer 2009;9:1-17.
43. Dou H, Shen R, Tao J, Huang L, Shi H, Chen H, *et al*. Curcumin suppresses the colon cancer proliferation by inhibiting Wnt/β-catenin pathways via miR-130a. Front Pharmacol 2017;8:877.