The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 1940-1949

Gene Expression of Effect of Combination Cassia Fistula Extract and Cisplatin on Inhibition of Human Colon Cancer SW480 Cell Line

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ABSTRACT

Background: There is a significant rise in the prevalence of colon cancer worldwide. A variety of therapeutic approaches have been tried, but none have been successful due to the aggressive spread of the tumor. Natural products derived from plants have traditionally been used in the medical field. **Objective:** The aim of the current study was to investigate the effect of Cassia Fistula cytotoxicity on a colon cancer cell line and the synergy between Cassia fistulae extract and cisplatin significantly to induce apoptosis through upregulation of p53 and Msh2.

Material and methods: Real-Time polymerase chain reaction indicated that gene expression proteins were likely involved in the pathway when the cytotoxicity potential of the plant extract on colon cancer cells was assessed using the (MTT) test. The database search techniques used with colon cancer cells identified the p53 gene and Msh2 gene's apoptotic function.

Results: Aqueous extract of the plant was most toxic to cancer cells. Further, p53 gene expression and Msh2 were observed to be high in cells treated with the plant extracts of C. fistula and $IC_{50}$of cisplatin (CDDP) +Cassia Fistula suggesting an involvement of mitochondrial proteins in the colon cancer cell apoptosis.

Conclusion: The results of the present study indicate that Cassia fistula extracts significantly regulate p53 and Msh2 expression to induce apoptosis. According to its cytotoxic and apoptotic action, Cassia fistula can be considered as a new discovery for the treatment of colon cancer.

Keywords: Colon cancer, Cell line SW480, Cassia Fistula, P53, Msh2, Experimental study, University of Thi-Qar.

INTRODUCTION

The Centers for Disease Control and Prevention (CDC) report that colorectal cancer (CRC) is one of the leading cancer-related deaths worldwide [(1)]. There is a possibility that colorectal cancer may be sporadic, but there are a number of internal factors (such as age, obesity, and inflammatory bowel disease) and external factors (such as smoking, inadequate fiber intake, exces/sive drinking, and eating a high-fat diet) that are associated with an increased risk of colorectal cancer (CC); colon and rectum [(2)].

It is believed that CC develops gradually due to various genetic and epigenetic changes accumulating over time. Chromosomal instability (CIN) linked to high-frequency aneuploidies and allele imbalances, is the primary cause of cancers (approximately 85% of them). The remaining 15% of individuals have faulty DNA mismatch repair (dMMR), frequently detected by microsatellite instability survey (MSI) or by testing for protein loss caused by DNA mismatch repair genesis detected by microsatellite instability survey (MSI) or by testing for protein loss caused by DNA mismatch repair genes, frequently MLHI, MSH2, MSH6, and PMS2. Sporadic CCs with dMMR have a better prognosis than non-dMMR CCs because they exhibit certain clinical and pathological characteristics, such as proximal colon predominance, poor mucosal histology and/or differentiation, infiltration of intraperitoneal and peritoneal lymphocytes, diploid DNA content [(3,4)].

The presence of an MSI-H tumorigenic phenotype and loss of MLHl and MSH2 protein expression are strongly compatible with each other [(5)]. Apoptosis is a critical cell death mechanism that can effectively inhibit the development and growth of tumors; As a result, it is often dysregulated in a variety of malignancies [(6)]. Epigenetic and epigenetic modifications in apoptotic pathways enable the increased proliferation brought on by genetic abnormalities, which ultimately permits uncontrollable tumor growth. The equilibrium between proliferation and apoptosis strictly regulates colonic homeostasis. The growth and advancement of CRC depend on upsetting this balance. Additionally, CRC tumors usually exhibit an increased apoptotic threshold, which adds to treatment resistance [(7)].

p53 mutations often occur in more than 50% of all tumors, making it the most frequently altered gene in human tumors. Tumor formation and progression typically involve disruption of p53 function [(8)].

In 50% of human cancers, the p53 gene is altered. When some types of DNA damage occur, it is likely that their main natural function is to direct cell cycle arrest in the G1 or G2 phase of the cell cycle and, when the damage is very high, to induce apoptosis [(9)]. Recent research has confirmed the anti-cancer properties of substances of natural origin [(10)]. Because of the expected chemotherapy benefits of natural products, their use has expanded. As sources of novel trace elements and anti-inflammatory and

Received: 25/08/2022
Accepted: 28/10/2022

anti-cancer activities, medicinal plants are frequently exploited in the production of pharmaceuticals. Although there are many tools for early diagnosis and cancer prevention strategies, herbal remedies and synthetic pharmaceuticals can still be offered. Plant medicines are frequently used as one of the main anti-cancer therapies [(11)]. In this work, in addition to examining the underlying mechanisms, we looked at the combined effect of Cassia fistula and cisplatin on colon cancer cells. By controlling the apoptosis gene signaling system, Cassia fistula improved the chemotherapy effect of cisplatin on colon cancer cells.

The aim of the current study was to investigate the effect of Cassia Fistula cytotoxicity on a colon cancer cell line and the synergy between Cassia fistulae extract and cisplatin significantly to induce apoptosis through upregulation of p53 and Msh2.

MATERIALS AND METHODS

Cassia Fistula Extract and cisplatin preparation

The research plant collected dried Cassia fistula fruits and seeds, cleaned them with distilled water, and then dried them in the air. According to the extraction was carried out using aqueous extracts [(12)].

Up to 20 grams of plant powder by weight should be added to a pyrex flask along with 100 milliliters of boiling distilled water and left to steep for 30 minutes. The extract was then centrifuged at 2500 r/min for 10 minutes after the beaker was heated up overnight with a continuous magnetic stirrer. The recovered extract was run through Whatman filter paper and allowed to sit for the night before being baked at 40 to 45 degrees. Purchased from Mylan Corporation in France, 50 mL vials of 1 mg/mL cisplatin were weighed out, diluted in the right amount of water to achieve a final stock concentration (1000 g/mL), and sterilized using a Millipore filter of 0.22 M.

Cell line, growth conditions and treatment

The National Iranian Cell Bank of the Pasteur Institute in Tehran, Iran, was where the SW480 cell line was acquired. It was cultivated using 10% fetal bovine serum and Biowest's RPMI1640 medium (FBS, Biosera, South America). For cell passaging, 1% TripsinEDTA (ATOCEL Corporation, Budapest) was applied and incubated once the cells had reached 80% confluence. Complete cell culture media (1% PS and 10% FBS) was then added. Penicillin and streptomycin were added to a solution to prevent the growth of a range of Gram-positive and Gram-negative bacteria**.**

In vitro cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cell growth after 24 hours. A 96-well microplate containing a cell line divided into five groups and MTT powder was added to the wells after being dissolved in PBS medium. Cisplatin was administered to Group 1 at various concentrations (15.60, 31.25, 62.5, 125, 250, and 500 g/ml) and to Group 2 at various concentrations (15.60, 31.25, 62.5, 125, 250, and 500 g/ml). **Group 3** received treatments with mixtures of IC50 cisplatin and concentrations of C. Fistula (15.60, 31.25, 62.5, 125, 250, and 500 g/ml), while Group (5) received treatments with a combination of cisplatin and c. fistula at equal concentrations. After 4 hours, dimethyl sulfoxide was added to the wells at 37°C of incubation, and the absorbance was measured at 570 nm.

Evaluation of Gene Expression P53 and Msh2 Using Real-Time PCR

Real-time PCR was used to measure the expression levels of p53 and Msh2 using the appropriate primers (**Table 1**). Colon cancer cells (2×10^6) were planted into Petri dishes and maintained at 37°C for 24 hours to ensure full adhesion in order to isolate the RNA. Cells were exposed to various concentrations of cisplatin, c. fistula, IC50 from C. Fistula and CDDP, and a combination of CDDP + C. Fistula extract, before being cultured for a further 24 hours. After 5-min incubation, cells were scraped using a scraper or pipette tip, washed with ice-cold PBS, and then dissociated by adding 1 ml of GENEizol reagent directly into the culture dish. Then 200 µl of chloroform was added and vortexed vigorously for 15 seconds, and centrifuged at 12,000 rpm at 4°C for 20 minutes. The mixture was separated into the lower organic phase and the upper RNA-containing aqueous phase. The upper aqueous phase was transferred to a new tube and 0.5 mL of 70% ethanol was added to precipitate the RNA and incubated for 10 minutes followed by centrifugation at 12,000 rpm at 4°C for 20 minutes. An RNA pellet was obtained after removing the supernatant. The RNA pellets were then allowed to dry for 5-10 minutes in a dry bath before being dissolved in DEPC-treated water. At 260 nm and 280 nm, the OD of the RNA was measured last. In addition, using the Bio-Rad synthesis kit, equal amounts of RNA at different concentrations were used to generate cDNA. SYBR was used in RT-PCR on Applied Biosystems to examine its relative expression (green). A comparative threshold cycle (CT) approach was used to quantitatively compare the expression of each gene. For each gene of interest, beta-actin served as a control. Each Ct value was normalized to the Ct value of β-actin RNA. For each gene, Relative gene expression was defined as 2-Ct, and final gene expression was expressed as 2-Ct ± SEM. All reactions were performed in triplicate and primer sequences are shown in **Table 1**. One reaction mixture for the RT-PCR reaction contains reagents such as SYBR (5 µl), cDNA (1 µg), endonuclease-free water (variable) and primer (forward) + reverse, 0.4 µl). The RT-PCR cycling conditions for p53 and Msh2 are 95°C (10

min), 95°C (15 seconds), annealing temperature (1 minute), 72°C (1 minute); Step 2-4 (40 cycles) and melt curve (depending on device protocol). Real-time PCR was used to evaluate p53 and Msh2 gene expression.

Table 1: Sequences of the primers used in real-time PCR assays.

Primers Sequence 5'- 3'
p53 F TGGCCATCTACAAGCAGTCA R GGTACAGTCAGAGCCAACCT
Msh2 F ACCAGCAGCAAAGAAGTGCT R AGGGCATTTGTTTCACCTTG
Actin F GCGAGAAGATGACCCAGAT R GAGGCGTACAGGGATAGC

Ethical approval: The project was approved by The Local Ethics Committee at Thi-Qar University in accordance with University Order No. 5647 on 05/24/2022.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate.

Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and SD, and independent sample t-test was used for comparison between groups. Actin was used as a reference gene to normalize gene expression levels during gene expression estimation using a delta/delta computational approach.

A two-sample t-test was used to compare the delta Ct of treated and control cells after normalization. Using the delta/delta arithmetic method, folds between the expression levels of treated and untreated cells were averaged. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

The yellow tetrazolium dye is converted to a purple formazan product by the interaction of the MTT assay with living cells. This study's main objective was to use the MTT assay to examine how a Cassia Fistula extract affected the rate of cell growth and proliferation. Figure 1 displays the results from a 24-hour analysis of all cells treated with cisplatin at various concentrations (0-500 g/ml). As the cisplatin concentration increased, a decline in cell viability was seen in the cell line. After treatment with the drug, cell survival was significantly lower than that of the untreated cell ($P<0.05$) in the concentration-dependent function. Compared to cells that were not treated in any way the results showed that the extracted cells had the lowest levels of viability. Hence, it was more toxic to cancer cells (**Figure 2**). The greatest decrease in cell survival was recorded at concentrations of 500, 250, 15.25 and 31.25 μg/mL of the plant extract against colon cancer cell line SW480.

The assay showed that the fistula extract had no effect on cell line survival at 125 μM. There were significant ($P<0.05$) compared to the control group (cancer cells without treatment) and at all concentrations (15.60, 31.25, 62.5, 125, 250 and 500 μg/mL), there were significant cytotoxic effects at all concentrations. The IC50 was cisplatin 380.

The greatest decrease in cell survival was recorded at a concentration of 500μg/ml ICCP+ plant extract against a colon cancer cell line (**Figure 3**). The combination of equal concentrations of cisplatin and Cassia fistula, treatment after 24 hours of incubation was significantly ($P<0.05$) compared to the control group (cancer cells without treatment) at all concentrations (15.60, 31.25, 62.5, 125, 250 and 500 μg/ml). In addition, the inhibitory concentrations achieved after treatment of Cassia fistula extract with cisplatin at different concentrations are shown in **Figure 4**. As the concentration of the combination increased, it was noticed that the cells' viability decreased proportionally. The survival rate of colon cancer cells after treatment with combination was considerably lower than the untreated cell ($P<0.05$) in concentration-dependent function. The lower percentage of survivors served as evidence for this. The combination had an impact that inhibited the colon cancer cell line SW480 from proliferating at high concentrations (500 g/ml).

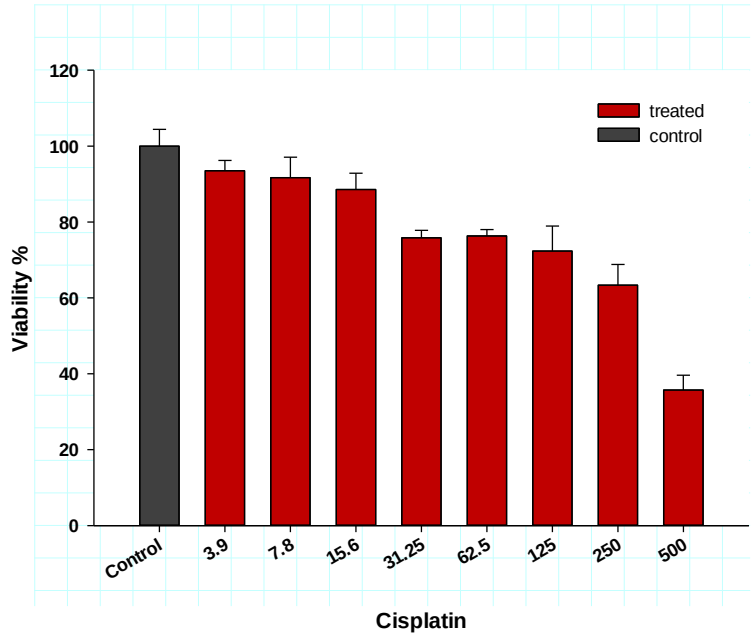

Figure (1): Effect of cisplatin on colon cancer cell viability after 24 hours. Cells were treated with cisplatin at concentrations of 15.60, 31.25, 62.5, 125, 250 and 500 g/mL and measured. The MTT assay was used to assess cell viability.

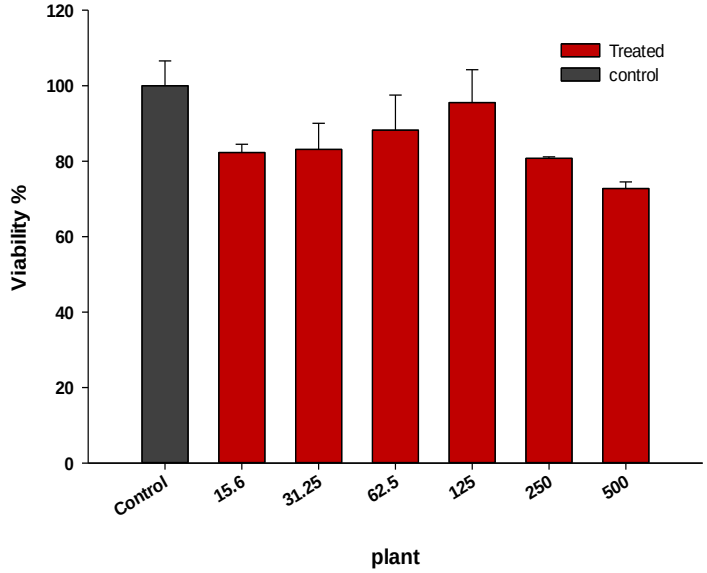

Figure (2): Effect of Cassia fistula on SW480 cell survival. Cell viability was assessed using the MTT assay after administration of the extract to cells at concentrations (15.60, 31.25, 62.5, 125, 250, and 500 g/mL).

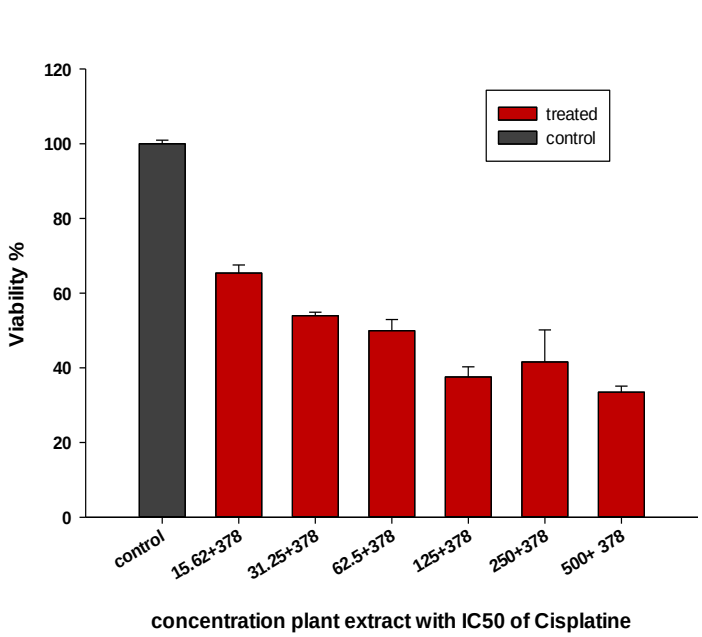

Figure (3): Effect of IC_{50} cisplatin and Cassia fistulae on the viability of SW480 cells. Cell viability was assessed using the MTT test 24 hours after cells were treated with the medication and extract at concentrations of (15.60, 31.25, 62.5, 125, 250, and 500 g/mL) and IC50, respectively.

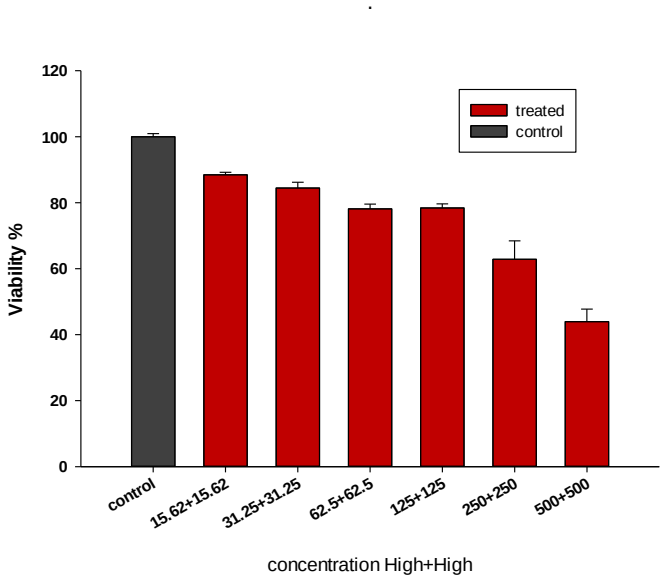

Figure (4): Cisplatin and Cassia fistulae have an effect on SW480 cells' survival ability. After 24 hours, cells were treated with high concentrations of the extract and the medication (15.60 + 15.60, 31.25 + 31.25, 62.5 + 62.5, 125 + 125, 250 + 250, and 500 + 500 g/mL). The MTT assay was used to determine cell viability.

Gene Expression Analysis

Effects of CDDP on the expression levels of P53 gene

The results indicated that treatment of colon cancer cell line SW480 with the drug (CDDP) leads to upregulation of p53 gene in the cell line compared to untreated cells (Fig. ml) compared with the other focus and control group. But there was no significance in cells treated with CDDP at a concentration of 500 µg/ml) compared to the untreated cell. Confirmation of changes in gene expression levels was in response to treatments.

Effects of C. Fistula on the expression levels of P53 gene

The colon cancer cell line SW480's apoptotic pathway was triggered by treatment with Cassia Fistula extract. The apoptotic mechanisms P53 and C.F. extract were shown to be active (**Figure 6**). Additionally, when compared to untreated cells, the expression level rose in the concentration (125g/ml), but there was no difference between other concentrations and the control group.

Effects of IC_{50} CDDP with C. Fistula on the expression levels of P53 gene The gene expression level change of P53 genes in response to treatment with IC50 of CDDP and C. F. extract were assessed Real-Time PCR. Results Real-TimePCR indicated that, treatment of SW480 cell line with IC50 of CDDP + C. F. extract leads to upregulation of P53 gene (Figure 7).The expression level of P53 gene in both plant extract with the drug utilized cell lines was upregulated significantly and increased in concentrations(31.25 and 62.5) µg /ml compared with another concentration and untreated cells. However, no significant between other concentrations with control group was found. Confirmation of the changes in gene expression levels was in response to treatments.

Effects of CDDP in combination with C. Fistula on the expression levels of P53 gene

Combined concentrations of the drug (CDDP) and C.F. extract were also tested on cell line. Results of cell counting after 24 hours are shown in **Figure 8**. It is noteworthy that all concentration of combination decreased the expression level of P53 compared with untreated cells also, did not have significant toxicity on cancer cells after being treated with (15.62,31.25,62.5,125,250 and 500µg/ml)concentration of combined of CDDP an extract with equal concentration.

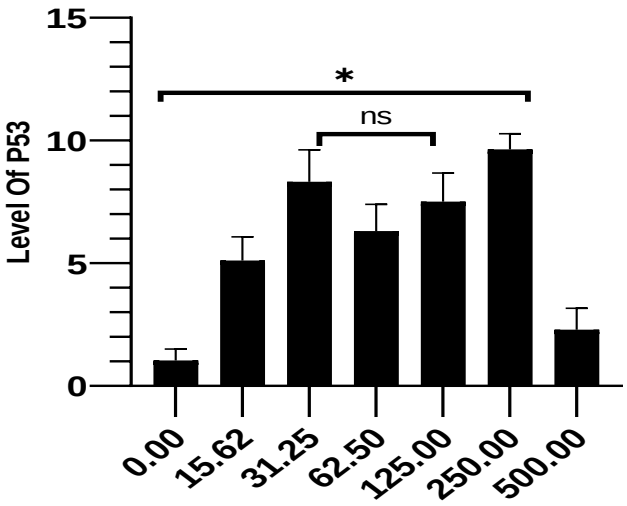

Figure (5): Changes in mRNA levels of P53 gene of colon cancer cell lines in response to treatment of cisplatin comparison with control. *P-value <0.05; NS non-significant.

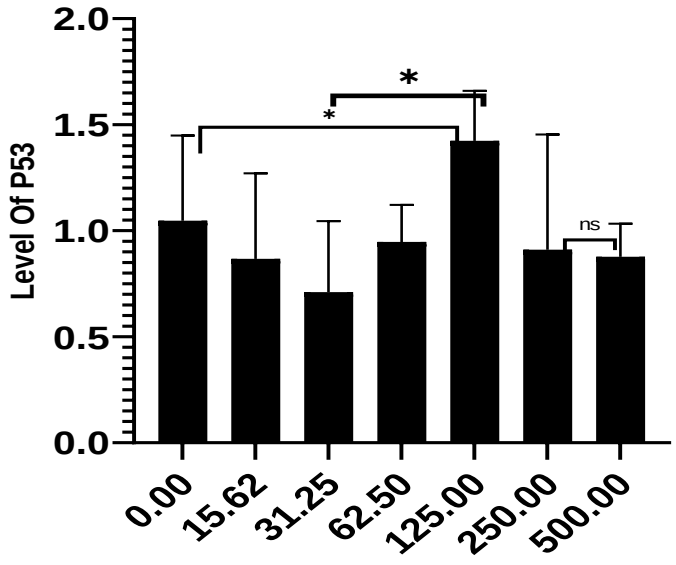

Figure (6): Changes in mRNA levels of gene P53 of colon cancer cell lines in response to treatment with Cassia Fistula comparison with control. *P-value <0.05; NS non-significant.

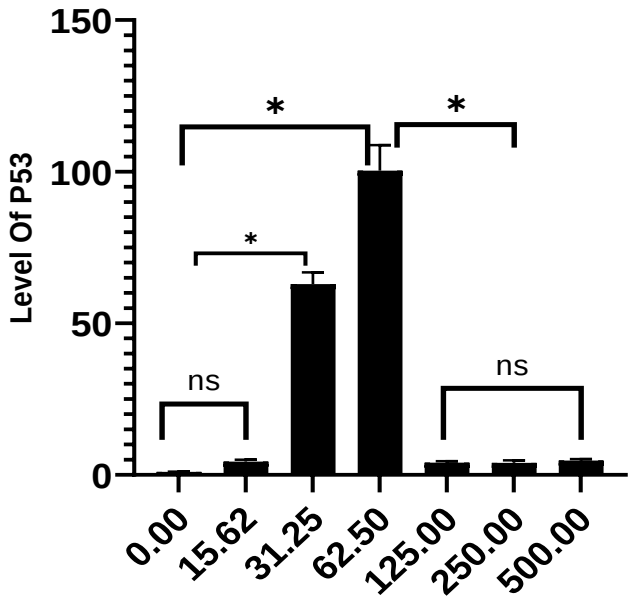

Figure (7): Changes in mRNA levels of P53 gene of colon cancer cell lines in response to treatment combination of cisplatin LC50 with Cassia Fistula comparison with control. *P-value <0.05; NS non-significant.

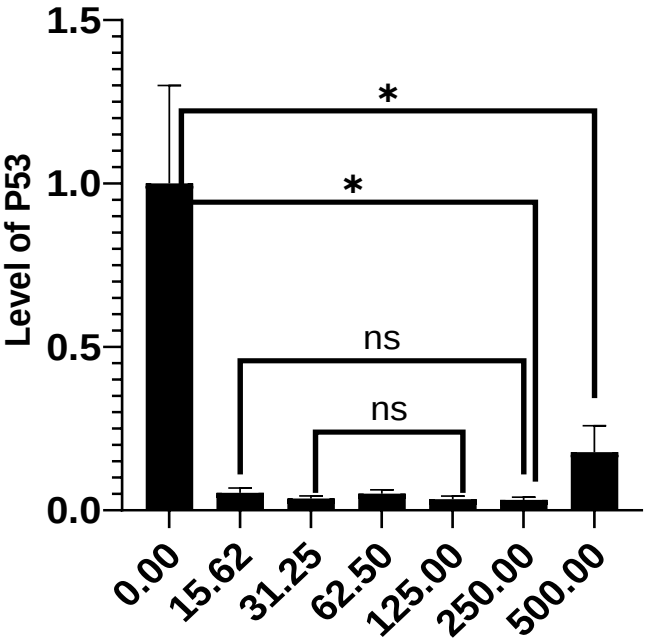

Figure (8): Changes in mRNA levels of P53 gene of colon cancer cell lines in response to treatment combination of cisplatin with Cassia Fistula comparison with control. *P-value <0.05; NS non-significant.

Results indicated that treatment of colon cancer cell line SW480 with drug CDDP leads to downregulation of Msh2 gene in cell line compared with untreated cells (**Figure 9**) showed expression level decreased in all concentration compared with untreated cells. Confirmation of the changes in gene expression levels was in response to treatments.

Effects of C. Fistula on the expression levels of MSH2 gene

The apoptotic pathway activated by treatment with Cassia Fistula extract in colon cancer cell line SW480, we found that C.F. extract activated Msh2, apoptotic pathways (**Figure 10**). Furthermore, expression level increased in concentration (125 and 250µg /ml) compared with untreated cells and other concentrations. Also there is significant between all concentrations compared with control group.

Effects of IC_{50} CDDP with C. Fistula on the expression levels of MSH2 gene

The gene expression level change of Msh2 genes in response to treatment with $IC5_0$ of CDDP and C. F. extract were assessed Real-Time PCR. Results indicated that, treatment of SW480 cell line with IC_{50} of CDDP + C. F. extract leads to upregulation of Msh2 gene (Figure 11).The expression level of the Msh2 gene in both plant extract with the drug utilized cell lines was upregulated significantly and increased in concentrations(31.25 and 62.5) µg /ml compared with other concentrations and untreated cells. Also increased in concentration (125,250 and 500) µg /ml compared with untreated cells but, no significant between concentration 15.52 µg /ml compared with untreated cells.

Effects of CDDP in combination with C. Fistula on the expression levels of MSH2 gene

Combined concentrations of the drug (CDDP) and C.F. extract were also tested on cell line. Results of cell counting after 24 hours are shown in **Figure 12**. It is noteworthy that all concentration of combination decreased the expression level of Msh2 compared with untreated cells**.**

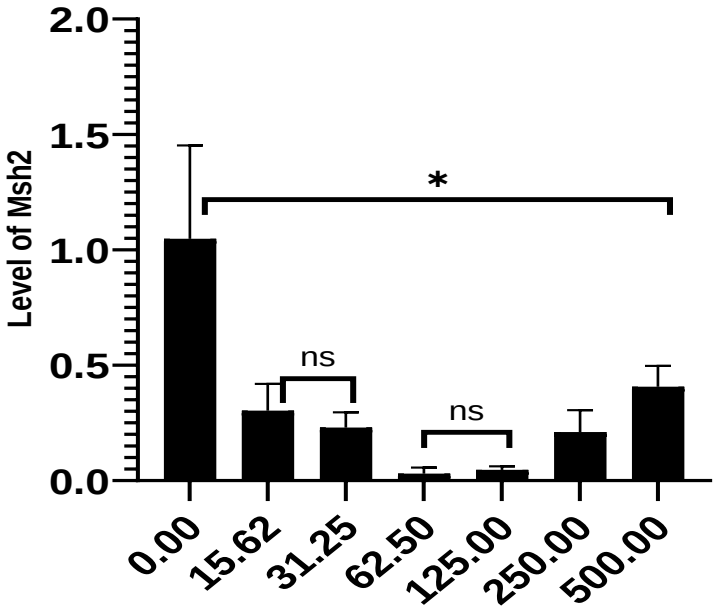

Figure (9): Colon cancer cell line: Msh2 gene mRNA levels have changed in response to cisplatin treatment as compared to the control group. *P-value <0.05; NS non-significant.

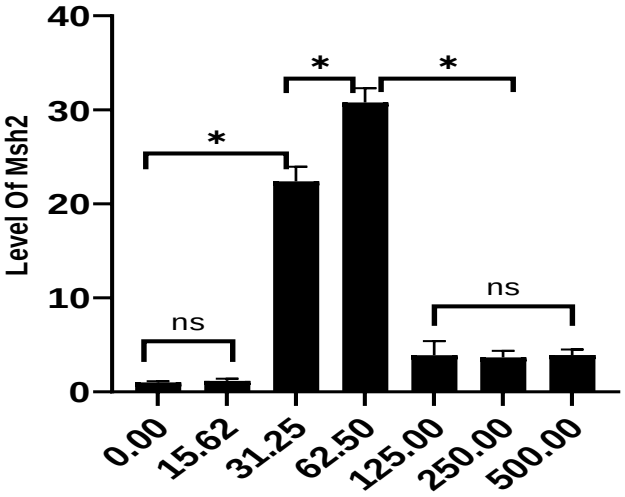

Figure (11): Colon cancer cell lines: Msh2 gene mRNA levels change in response to LC50 cisplatin and Cassia fistula therapy when compared to the control group. *P-value <0.05; NS non-significant.

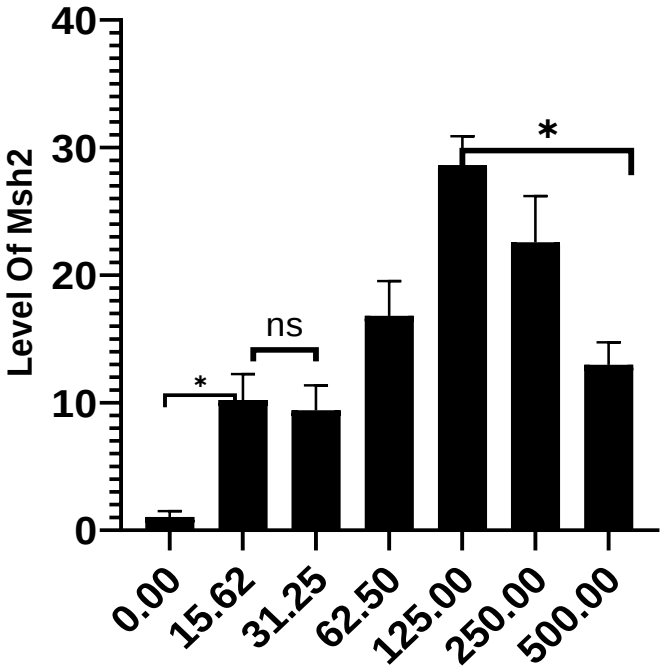

Figure (10): Msh2 gene mRNA levels in colon cancer cell line are altered in response to treatment by comparing Cassia fistula with the control group. *P-value <0.05; NS non-significant.

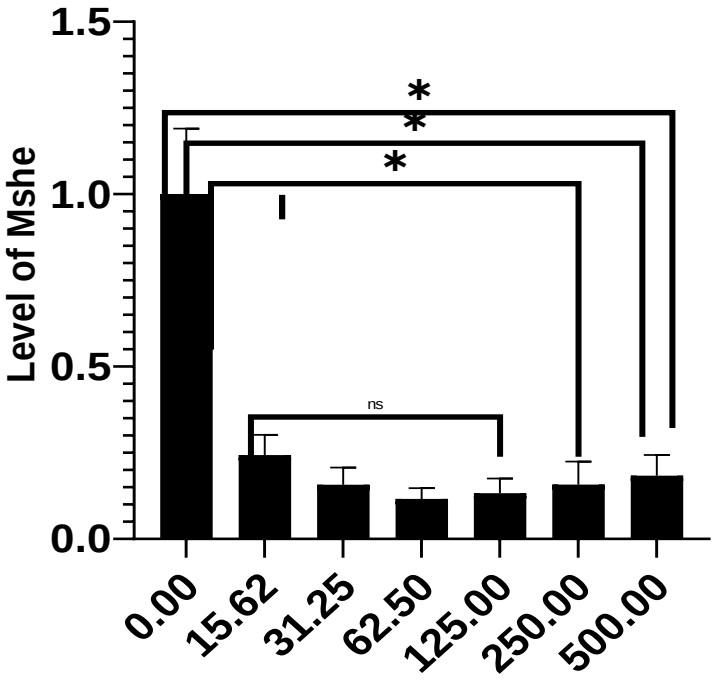

Figure 12. Changes in colon cancer cell line Msh2 gene mRNA levels in response to cisplatin and a Cassia fistula combined therapy as compared to control. *P-value <0.05; NS non-significant.

DISCUSSION

Many of the research that is now being conducted as part of the process of creating effective methods of cancer prevention mainly focuses on the use of naturally occurring bioactive substances are capable of eliciting selective apoptosis in cancer cells [13]. Researchers who study cancer typically agree on the positive effects of combining the natural extract with anticancer drugs [14]. Currently, several studies have found that natural medicinal compounds derived from plants play an important role in the treatment of human diseases [29] and that the use of these compounds is an effective way to treat metastatic cancers [15,16]. According to reports, using natural items during chemotherapy can enhance its effects [17,18]. In-depth investigations are currently being conducted to reevaluate the therapeutic potential of natural compounds. This happens as a result of natural products' improved effectiveness and their low side effects [19].

This current study set out to explore the potential effectiveness of combination chemotherapy. However, there is a chance of toxicities building up, thus it is frequently important to work with low dosages of both medications to prevent experiencing negative side effects. The Cassia fistulae aqueous extract was tested against a colon cancer cell line to determine its activity. However, a number of sources have already described citronellol and linoleic acid as potential anti-cancer drugs [20,21], which produced cell cycle arrest at various phases [6]. Our investigation showed that the plant material's aqueous extract has anticancer properties when tested against the colon cancer cell line SW480. The results for cytotoxic effects values (**Figures 1, 2, 3 and 4**) demonstrated this. The MTT test findings demonstrated that the extract's antiproliferative abilities follow a concentration-dependent pattern.

At a concentration of 500 g/ml, the plant extract showed the greatest anti-proliferative effect on colon cancer cells. These effects were seen in cells treated with cisplatin, fistula cassia, IC50 from CDDP+ extract, and cells treated with a combination of the three. The anti-proliferative effects of the plant extracts on the cancer cells used are likely due to the oxidative actions of the plant's phytochemical components. Increased oxidative stress in cancer cells is what gives phenol-containing phytochemicals their antiproliferative properties. This is done by blocking ROS scavenging systems, deactivating pro-survival signals, activating signals associated with apoptosis, inducing DNA damage, and inhibiting signaling pathways that promote cancer cell proliferation [22]. Although they used different cancer cell lines, components, and solvents, the results are consistent with those of the experiment [23]. The extract may have an effect on cell membrane integrity and cell disruption at different stages. Highly compressed nuclei and broken chromatin were present in tumor cells exposed to methanol extract of fistulae. This could be caused by the formation of apoptosis or fragmentation of genomic DNA [23]. Our findings indicate that Cassia fistula extracts have a specific effect on the regulation of p53 and Msh2 in a colon cancer cell line. Msh2 gene expression and p53 up-regulation work in a concentration-dependent manner. In this study, the expression level of p53 gene in Cassia fistula-treated cells was higher than in cells of the control group. Compared with the untreated cell line, Msh2 expression was similarly elevated in cells treated with plant extracts. These alterations show that Cassia's fistula can cause apoptosis, and real-time PCR data show that Clostridium fistulae have an increased rate of first apoptosis compared to the control group. This distinction is important and shows that this plant significantly influences apoptosis. Other research has shown that the tumor suppressor protein p53 is a transcription factor constitutively active in response to a range of stressors [24]. Epiafzelechin-treated MG-63 cells had higher levels of p53.

Researchers Fu and colleagues found that tannins and flavonoids from the rhizome of Smilax china L. were able to inhibit the growth of A549 human lung cancer cells and induce apoptosis. The expression of p53 and p-p53 proteins was increased to achieve this [25].

In another study, P. oleracea extracts were found to significantly increase apoptosis in PANC-1 cells while retaining a lower level of necrosis. In general, it appears that after treatment of a cancer cell with a plant extract, more apoptosis than necrosis occurs. Consistent with previous findings from studies using MTT, flow cytometry, and microscopic observations, analysis of gene expression in a cancer cell line revealed a significant decrease in CDK gene expression. In addition, these phytochemicals have the ability to either upregulate or downregulate the expression of apoptosis-inhibiting proteins including p53 and Bcle2 in a range of cancer cell lines and animal models, as well as activate caspases and other pro-apoptotic molecules. Currently understood information indicates that Cassia fistula leaves have hepatoprotective properties [27].

The human colon cancer cell line COLO 320DM was subjected to dose- and time-dependent cytotoxicity by apoptosis from an ethyl acetate extract of flowers containing rhein [28]. Mice exposed to DENA-induced hepatotoxicity responded positively to the hepatoprotective effects of Cassia fistula leaf extracts [29]. In our study, the combination of Cassia fistula extract and cisplatin enhanced the antitumor effect of cisplatin on tumor growth inhibition, consistent with previous findings [17]. An important protein for inducing apoptosis after exposure of cells to cisplatin is p53. Furthermore, there is some evidence that p53 protein mutations are associated

with abnormal expression of the MMR protein MSH2 [30], and other MMR proteins [31]. In addition, p53 is believed to be involved in MMR and thus in mechanisms associated with DNA damage tolerance [32]. By contrast, Brown and Wouters [33] reported that neither the status of p53 nor the ability of cells to undergo apoptosis appears to play an important role in the sensitivity or resistance of these cells to DNA-damaging agents.

The results of our study showed that Cassia fistula extract and combination of IC50 CDDP + Cassia fistula extract increased Msh2 expression compared to the control group and it increased in concentration 31.25 and 62.5 μM (**Figure 10**). There was no significant change in the treated cell group with CDDP alone (**Figure 11**) in another previous study [34].

In response to 5FU clinical findings also suggesting a deficiency in MMR that reverses the antitumor effect, cellular studies support a role for the MMR system in general and for MSH2 in particular for 5FU. The MMR protein MSH2, which is the most abundant, initiates repair and cell signaling in response to DNA damage [35]. Therefore, it is reasonable to assume that MSH2 is an important MMR protein in cell signaling. Researchers using cell lines have established an inverse dose-response association between MSH2 and DNA tolerance demonstrated a positive correlation between MSH2 gene expression and survival [33].

CONCLUSION

Using real-time PCR, the present study aimed to evaluate the potential effect of Cassia fistula on the induction of apoptosis in the colon cancer cell line SW480. This indicates that treatment with Cassia fistula significantly affects the apoptosis of a colon cancer cell line. Therefore, these results can be applied to future knowledge on how plants regulate apoptosis. Plant extracts can be used at the correct concentration instead of or in addition to existing cytotoxic chemotherapy regimens. The mechanism of action and characterization of Cassia fistula aqueous extract could be a subject of future research. To achieve a good outcome, it was necessary to carry out those molecular research to investigate potential apoptotic pathways and to evaluate the cytotoxic activity of Cassia fistula.

Conflict of interest: nil**.**
Funding: nil.

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