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Celecoxib inhibits cancer growth through cyclooxygenase 2 (COX2) independent pathways in HepG2 hepatocellular carcinoma

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ABSTRACT

Chronic inflammation has long been associated to carcinogenesis. Evidence has shown that nonsteroidal anti-inflammatory drugs (NSAIDs) may decrease the risk of certain types of cancer. In this study, we assessed the anti-proliferative effects of different NSAIDs against liver cancer using HepG2 cell line. In addition, we evaluated the combined effects of a selective COX2 inhibitor (Celecoxib) with chemotherapeutic drugs. The anti-proliferative and combined effects of COX2 inhibitors were evaluated by MTT assay. The effect of COX2 inhibitors on gene expression was assessed using real time PCR (RT-PCR). Also, the effect of COX2 inhibitors on colony formation was assessed through colony-formation assay. COX2 inhibitors treatment significantly inhibited the growth of HepG2 cells in a dose-dependent manner. The combined treatment of Celecoxib with either doxorubicin or raloxifene resulted in synergistic effects. In addition, Celecoxib treatment significantly reduced the expression of Bcl2 and VEGF genes in HepG2 cells, while the COX2 gene was not detected at all in HepG2 cell line suggesting that the anti-tumorigenic activity of Celecoxib is COX2 independent. Our results also revealed that COX2 inhibitors treatment significantly reduced the number and size of colonies formed by HepG2 cells.

Keywords: Hepatocellular Carcinoma, COX2 inhibitors, Celecoxib.

INTRODUCTION

Cancer is a general term that describes the state where cells at a certain part of the body grow and reproduce in an uncontrolled manner (1). Nowadays, cancer is considered one of the major causes of death around the world. It has moved from the third most common cause of death in 1990 to the second most common cause of death in 2013 next to heart disease (2).

Liver cancer or Hepatocellular Carcinoma (HCC) is one of the most frequent malignancies worldwide. Liver cancer is the second most frequent cause of death from cancer globally (3) and it's the sixth most commonly occurring cancer. Mainly, the risk factors for HCC are rolling around any agent leading to chronic hepatic injury, these factors can be summarized into the following: Hepatitis B virus (HBV) that accounts for 50% of all HCC cases and Hepatitis C virus (HCV) accounting for 25% of all cases of HCC (4). Although there are advancements in the treatments of HCC, its prognosis is still poor due to the recurrence and metastasis (5). While surgical resection and transplantation are the cornerstones of therapies in the early stage of the disease, regional therapy, and Sorafenib are beneficial in those with more advanced disease (6,7).

Cyclooxygenase 2 (COX2) is one of the molecular targets that have been increasingly investigated in many cancer types such as lung, colorectal, and breast cancer (8). It was shown that COX2 may have effects on many processes that are involved in different stages of carcinogenesis including angiogenesis, apoptosis, immune

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function, tumor growth, and invasiveness (8). Several studies illustrate the strong relationship between COX2 enzyme overexpression and Hepatocellular Carcinoma progression and grading and may give an idea about the role of this protein in hepatocarcinogenesis (9).

NSAIDs are of the most used groups of drugs worldwide to treat inflammation and pain. However numerous side effects are reported due to the nonselective action on both COX1 and COX2 enzymes (10). The more selective drugs that are called Coxibs or selective COX2 inhibitors have higher selectivity toward COX2 enzymes and low affinity toward COX1 (11). It has been demonstrated that COX2 inhibitors suppressed the growth of the HCC cell lines in a time and dose-dependent manner especially for cells expressing COX2 protein (12).

In this study, we aimed to investigate the expression pattern of COX2 protein in Hepatocellular Carcinoma, especially the HepG2 cell line, and to determine its clinical significance. Besides, the anti-tumorigenic effects of selective COX2 inhibitors against Hepatocellular Carcinoma were evaluated.

Materials and Methods

Human Hepatocellular Carcinoma cell line HepG2 and human Fibroblast cells were purchased from American Type Culture Collection (ATCC, USA), Celecoxib, Etoricoxib, Indomethacin, Aspirin and Raloxifene hydrochloride were all from (Sigma-Aldrich, USA), Doxorubicin hydrochloride 2mg/ml (Ebewe Untrerach, Austria), Direct-zol to RNA (miniprep KIT, USA), Primescript to Master mix KIT (Takara, Japan), PCR TB Green Premix Ex Tag to 2 (Tli RNase PLUS) KIT (Takara, Japan).

HepG2 cell line was cultured in DMEM culture medium supplemented with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 2 mM L-glutamine, 1% non-essential amino acid, and 1% sodium pyruvate. Cells were incubated in 5% CO_2, 95% humidified air at 37°C.

Cell Viability Assay

Anti-proliferative activity of drugs on the HepG2 cell line was assessed using MTT assay 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay as previously described (13). HepG2 cells were plated in 96-well plates at concentration 8000 cells per 100 µl in DMEM medium and incubate at 37°C, after 24 h the media was discarded and the cells were treated with drugs with increasing doses of 50-350 µM for Etoricoxib, Indomethacin, and Aspirin, and 30-160 µM for Celecoxib conditions. After treatment addition for 24 h, 48 h, and 72 h incubation periods 10 µl MTT dye at concentration 5 mg/ml was added for each well. The optical density was measured at 570 nm and 630 nm wavelength using a microplate reader (µ Quant Plate Reader, Biotek, USA).

Drug Combination with Doxorubicin and Raloxifene

Hepatocellular Carcinoma cell line HepG2 was seeded in 96-well plates (2 replicate/group) for 24 h, to observe the combined outcome of Celecoxib on Doxorubicin and Raloxifene. Cells were treated with a range of concentrations of Celecoxib, Doxorubicin, and Raloxifene alone and in combination. The combination ratios of doxorubicin with Celecoxib were (1:5,1:20 and 1:50), the combination ratios of Raloxifene and Celecoxib were (1:5, 1:10, and 1:20). After 24, 48, and 72 hours of treatment MTT dye was added, and viability was measured. The Combination Index (CI) was calculated using Compusyn software based on the Chou-Talalys combination index theorem.

$$CI = \frac{D1}{DX1} + \frac{D2}{DX2}$$

Where (Dx)1 =dose of drug 1 to produce 50% cell kill alone, (D)1 = dose of drug 1 to produce 50% cell kill in combination with (D)2. (Dx)2 = dose of drug 2 to produce 50% cell kill alone, (D)2 = dose of drug 2 to produce 50% cell kill in combination with (D)1. The Combination Index CI values for combined treatment are shown in Table 2. Overall CI < 1 indicates a synergistic effect, while CI=1 signifies additive effect and CI >1 means antagonistic effect.

Colony Formation Assay

HepG2 cells were seeded in 6 well plates and could attach over the night. After 24 h of seeding each well was treated with one of the following: Celecoxib IC_{50}, Celecoxib half IC_{50}, Etoricoxib IC_{50}, Etoricoxib half IC_{50}, and untreated control wells containing only media. After 48 h of treatment, the media was discarded, the cells were detached by trypsin and counted using a hemocytometer. To prepare for the assay, two layers of agar were prepared, the first layer consisted of equal volumes of 2X media and 1% agar. After preparation of this mixture 2 mL of it was added to each well and allowed to solidify. The upper layer consisting of 1mL of 0.6% noble agar and a total of 1 ml of medium containing cell pellets that were previously collected and left to solidify. After that, the plate was incubated at 37°C in a humidified incubator for 2-3 weeks and was monitored every other day to see the colonies' growth, photos were taken after 20 days of incubation using an Evos microscope.

Extraction of Cellular RNA and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RNA extraction was carried out according to Direct-Zol TM RNA Miniprep kit instructions using Triazol reagent. Subsequently, cDNA was synthesized using Primescript TM RT Master Mix kit instructions to produce the cDNA. Reverses Transcriptase - Polymerase Chain Reaction (RT-PCR) was performed using TB Green Premix kit using 4 primers *Bcl-2*, *VEGF*, *COX2* and *GAPDH* with its forward and reverse sequences, as previously described (14).

RESLTS

Effect of COX Inhibitors on the Viability of Hepatocellular Carcinoma Cell line HepG2.

To examine the effects of COX inhibitors (Celecoxib, Etoricoxib, Indomethacin, and Aspirin) on the viability of HepG2 cell line, MTT assay was carried out, and the results are shown in Figure 1. All drugs were tested in three independent experiments after 24 h, 48 h, and 72 h incubation periods. According to the results, Celecoxib significantly inhibited the growth of cells compared to the untreated control cells in a dose and time-dependent manner. Other COX inhibitors also yielded inhibition in growth but with higher 50% inhibitory concentrations (IC_{50}). The anti-proliferative effect of other COX inhibitors significantly varied. Etoricoxib has IC_{50} of 260.9 µM after 48 h, and 310.1 µM after 72 h incubation time. Aspirin always has the highest IC_{50} values among the four examined drugs indicating the worst activity.

Effect of Chemotherapeutic Agents on the Viability of HepG2 Cell Line

The effect of known chemotherapeutic agents such as Doxorubicin and Raloxifene on the viability of HepG2 cell line after 24 h, 48 h, and 72 h is shown in Figure 2. Treatment of HepG2 cell line with Doxorubicin in a concentration range of 1.5-10 µM obviously inhibited the cell viability in a time and dose-dependent manner. This result was also observed upon the treatment of HepG2 cells with Raloxifene in a range of 12.5-200 µM. Such treatment yielded growth inhibition in a time and dose-dependent manner. The 50% inhibitory concentration (IC_{50}) values for the examined chemotherapeutic agents Doxorubicin and Raloxifene against HepG2 cell line are shown in Table

Effect of Combined treatment of Celecoxib and Chemotherapeutic Agents on HepG2 Cell Line

This study evaluated the combined effect of COX2 selective inhibitor Celecoxib and standard chemotherapeutic agents of Doxorubicin or Raloxifene. Cells were treated with Celecoxib and Doxorubicin at combination ratios of 1:5,1:20, and 1:50. The results revealed that treatment with Celecoxib and Doxorubicin in all combination ratios significantly decreased cancer cell viability compared to treatment with Doxorubicin alone, as shown in Figure 3, and this combination reduced the IC_{50} of Doxorubicin on HepG2 cells by 2 folds. Besides, treatment of HepG2 cells with Raloxifene and Celecoxib at combination ratios of 1:5, 1:10, and 1:20 reduced cell

viability when compared to Raloxifene treatment alone in the first 24 h, as shown in Figure 3, and this decreased the IC_{50} of Raloxifene on HepG2 cells by almost 4 folds.

Effect of COX2 Selective Inhibitor Celecoxib on Genes Expression.

The effects obtained from the treatment of HepG2 cells with selective COX2 inhibitor Celecoxib on gene expression were investigated with RT-PCR experiment upon the use of four genes; *GABDH* as a housekeeping gene, and three examined genes including *Bcl2*, *VEGF*, and *COX2*. The results showed that untreated HepG2 cells express the *Bcl2* gene, an anti-apoptotic gene, at a level that is 75% higher than the level of expression in the control fibroblast cells, a model for normal cells. Upon the treatment of HepG2 cells with 0.1 IC_{50} and 0.25 IC_{50} of Celecoxib, the relative gene expression of *Bcl2* was changed according to the concentration of drug used. Interestingly, 0.1 IC_{50} resulted in a 25% reduction in Bcl2 gene expression relative to the untreated HepG2 cells. On the other hand, treatment with 0.25 IC_{50} yielded a 40% reduction in *Bcl2* gene expression relative to the untreated HepG2 cells, as shown in Figure 4 (A). Also, the basal level of *VEGF* gene expression, an important gene for angiogenesis and vascularization of tumor cells, was pronounced in HepG2 untreated cells with more than 25% relative to the basal gene expression in fibroblast cells. When treated with 0.1 IC_{50} and 0.25 IC_{50} of Celecoxib, the reduction in gene expression was 55% and 50% respectively Figure 4 (B). Remarkably, the *COX2* gene was not detected at all in HepG2 cells while it was detected in Fibroblast cells. This result may indicate that different pathways that are COX2 independent may be involved in the observed anti-proliferative activities of Celecoxib.

Effect of COX Inhibitors on Colony Formation in HepG2 cells

Colony Formation Assay(CFA) is an in vitro cell survival assay that depends on the ability of separate cells to grow up into colonies (14). The results demonstrated that cells treated with IC_{50} of Celecoxib yielded fewer colonies compared to the control untreated HepG2 cells, and these colonies were smaller in size than the colonies in the control well. Regarding cells treated with half IC_{50} of Celecoxib, although a lower number of colonies was noticed when compared with the control wells, the effect on size was less obvious than the effect of a higher concentration of Celecoxib, as shown in Figure 5(A). Concerning Etoricoxib, its IC_{50} concentration affected the number of colonies significantly with fewer colonies compared to the control group, but its effect on size was less pronounced than the effect of Celecoxib on size. Half IC_{50} of Etoricoxib produced less effect than its IC_{50} with a lower number of colonies than the control but more than the of IC_{50} Etoricoxib as shown in Figure 5(B).

DISCUSSION

Several studies illustrated the strong relationship between COX2 enzyme overexpression and hepatocarcinogenesis, indicating the role of this enzyme in Hepatocellular Carcinoma progression and grading (9). According to the available data, the regulation of the COX2 signaling pathway was a vital source to try new drugs and new therapeutic approaches on HCC (15).

The present study has demonstrated that selective COX2 inhibitors Celecoxib significantly inhibited the growth of HepG2 cells compared to untreated control cells, with IC_{50} of around 55 µM and this finding is consistent with other studies (8), (12). COX inhibitors induced cell death in a concentration-dependent manner in HepG2 cells. In addition, compared to literature, it was reported that COX inhibitors, specifically COX2 selective inhibitors exerted their anti-proliferative effect on various cancer types like esophageal squamous cell carcinoma (16), and Familial adenomatous polyps (17).

In this study, Celecoxib was the most active drug among the tested drugs, while Aspirin was the least potent one. Such a result can be attributed to the fact that Celecoxib may work through different mechanisms of action on cancer cells and not only targeting COX2

enzymes, such mechanisms may include modulating of gene expression as was reported in different studies (18). Dai *et al*. reported that Celecoxib may exert its anti-tumor activity by affecting PNO1 expression and interfering with the AKT/mTOR signaling pathway in hepatocellular carcinoma (HCC) (18).

Traditional methods of cancer treatment have limited success due to systemic side effects, development of drug resistance, and sub-optimal drug concentration at the tumor site (19). Multi-target inhibitors or formulations are an attractive alternative to traditional therapy which synergistically inhibit multiple pathways that are essential for the growth of cancer cells. This study demonstrated the beneficial combined effects of Celecoxib with Doxorubicin and Raloxifene. Doxorubicin is a type of chemotherapeutic agents called anthracyclines. It slows or stops the growth of cancer cells by blocking an enzyme called Topoisomerase 2, Doxorubicin is given intravenously and used to treat multiple cancers like Breast, lung, and multiple myeloma (20). However Multiple cytotoxic effects on multiple organs and tumor resistance limit its clinical uses (21). Besides, Raloxifene is a selective estrogen enzyme modulator that induces an antagonistic effect on estrogen enzyme in breast tissues (22), Raloxifene has been approved by FDA for prevention and treatment of invasive estrogen enzyme-positive breast cancer in postmenopausal women, but it also increases the risk for venous thromboembolism (23). According to these results, the beneficial effect of combined treatment may be due to enhance the anti-proliferative effect of the chemotherapeutic agent, reduce the resistance, and decrease side effects by using a less concentration of such drugs while maintaining the same effectiveness.

The synergistic effect between Doxorubicin and Celecoxib has been reported before in skin cancer, and the researchers believed that this effect was due to the inhibition of multiple key signaling pathways like protein kinase B (AKT) which is a mediator of signal transduction that has a central role in tumorigenesis and cancer development and COX2 expression (24). The mechanism of synergistic inhibition of these drugs on Hepatocellular Carcinoma is not completely clear and needs further investigation. Regarding Celecoxib and Raloxifene, a synergistic inhibition effect has been detected after 24 h of treatment. Raloxifene alone may produce an apoptotic effect on HepG2 cell through activation of the Aryl hydrocarbon enzyme (AhR) signaling pathway that leads to apoptosis in HepG2 cells according to O'Donnell *et al.* (2014). The synergistic inhibition between Celecoxib and Raloxifene on Hepatocellular Carcinoma has not been studied before, so our study is the first report validating this unique combination.

P- glycoprotein is overexpressed in many cancer cells, it could extrude excessive effect chemotherapeutic drugs from cells and prevent the cytotoxic effect. It was reported that Celecoxib reduced p-glycoprotein expression and this produced an apoptotic effect on cells (26), also some studies stated that Raloxifene increases the P-glycoprotein ATPase activity and accordingly it will decrease the p-glycoprotein activity (27), so the synergistic effect between Celecoxib and Raloxifene may be through the activity on p- glycoprotein.

The mechanism of anti-cancer activity of Celecoxib has been described via COX-dependent and independent pathways (28),(29). In this study, the effect of Celecoxib on gene expression on three genes was studied *Bcl2*, *VEGF-A*, *COX2*, and *GABDH* as a housekeeping gene.

Bcl2 was the first anti-death gene discovered. Bcl2-family protein regulates all types of cell death including apoptosis, necrosis, and autophagy. Overexpression of this gene is related to anti-apoptotic proteins and demonstrated to inhibit cell death. According to our results, it can be concluded that Celecoxib induced apoptosis to HepG2 through the reduction of Bcl*2* gene expression**.** The effect of Celecoxib on modulating *Bcl2* gene expression has been reported before (30) (31), and other data demonstrated that Celecoxib induced apoptosis through

reducing the level of the anti-apoptotic protein Bcl2 in H22 hepatoma cells (32), however, this is the first time to report such finding in HepG2 cell line.

Concerning the *VEGF-A* gene, it is the most prominent and well-researched regulator of angiogenesis in cancer cells (33). It was reported that Celecoxib inhibits vascular endothelial growth factor expression and reduces angiogenesis in human pancreatic cancer (34). The reduction of *VEGF* gene expression in Hepatocellular Carcinoma's HepG2 cell line is studied for the first time here.

Results regarding Cyclooxygenase 2 (*COX2)* gene expression were interesting, COX2 was not detected at all in HepG2 cells, but it was detected in Fibroblast cells. Our results are consistent with previously reported data, which concluded that HepG2 cell line does not contain *COX2* genes(35). Accordingly, it can be concluded that the effect of Celecoxib on HepG2 cells is independent of the COX2 enzyme.

Clonogenic assay or colony-forming assay enables an assessment of the difference in proliferative capacity between control untreated cells and cells that have undergone various treatments, to provide a further measure of cell death (36-38). This assay can yield important information about the long-term proliferative potential of cells that cannot be determined by short-term assays.

In this study, HepG2 was divided into groups, and each group was subjected to different treatment, IC_{50} of Celecoxib, half IC_{50} of Celecoxib, IC_{50} of Etoricoxib, and half IC_{50} of Etoricoxib and control group. Celecoxib IC_{50} decreases the number and size of colonies formed by HepG2 cells significantly. Half IC_{50} of Celecoxib decrease the number of colonies but its effect on size was less obvious than IC_{50} of Celecoxib, all these effects were compared to untreated HepG2 cells. Regarding Etoricoxib, its IC_{50} effect on colony formation in HepG2 cells was pronounced, it decreased the number and size of colonies formed by HepG2 compared to untreated cells, half IC_{50} of Etoricoxib also produced colonies with less size and number than control but higher than IC_{50} well.

CONCLUSION

COX inhibitors (Celecoxib, Etoricoxib, Indomethacin, and Aspirin) inhibited the growth of HepG2 cells in a concentration and time-dependent manner. Celecoxib was the most powerful agent and Aspirin was the least potent. Celecoxib anti-proliferative activity is mediated through the induction of apoptosis by downregulation of the Bcl2 gene. Combination of Celecoxib with Doxorubicin resulted in synergistic inhibition of HepG2 cell viability at all incubation periods 24 h,48 h, and 72 h, while the combination with Raloxifene resulted in a synergistic effect after 24 h. COX inhibitors, Celecoxib and Etoricoxib, significantly lower the size and number of colonies formed by HepG2 cells. Celecoxib modulated gene expression in HepG2 cell line, it decreased the expression of Bcl*2* and *VEGF-A* genes. Most interestingly, the COX2 gene wasn't detected in HepG2 cell line suggesting a COX2 independent pathway that is involved in the observed COX2 inhibitor anti-proliferative activity.

Figure 1A

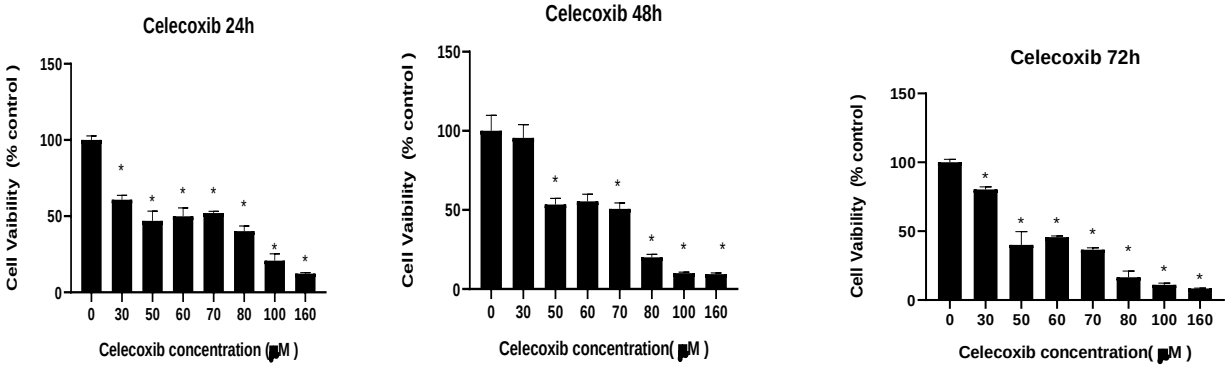


Figure 1: Effect of COX inhibitors on HepG2 cells viability.

(A) Effect of Celecoxib on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Figure 1B:

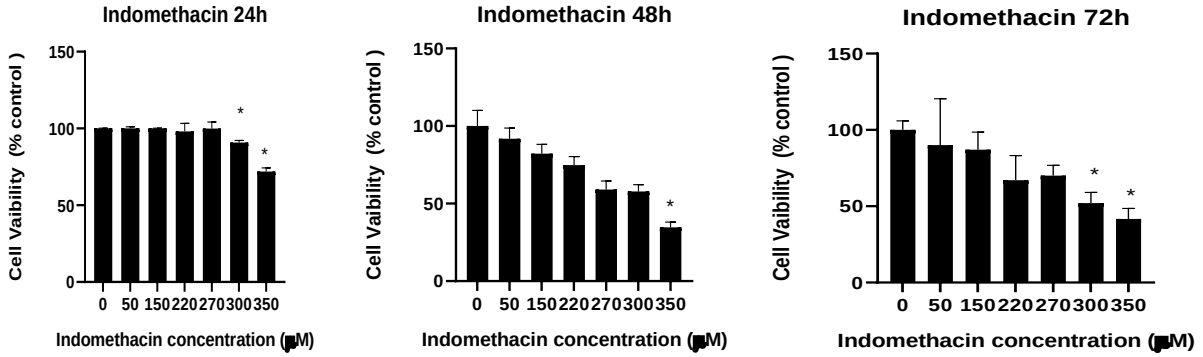


Figure 1: (continued) Effect of COX inhibitors on HepG2 cells viability.

(B) Effect of Indomethacin on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Figure 1C:

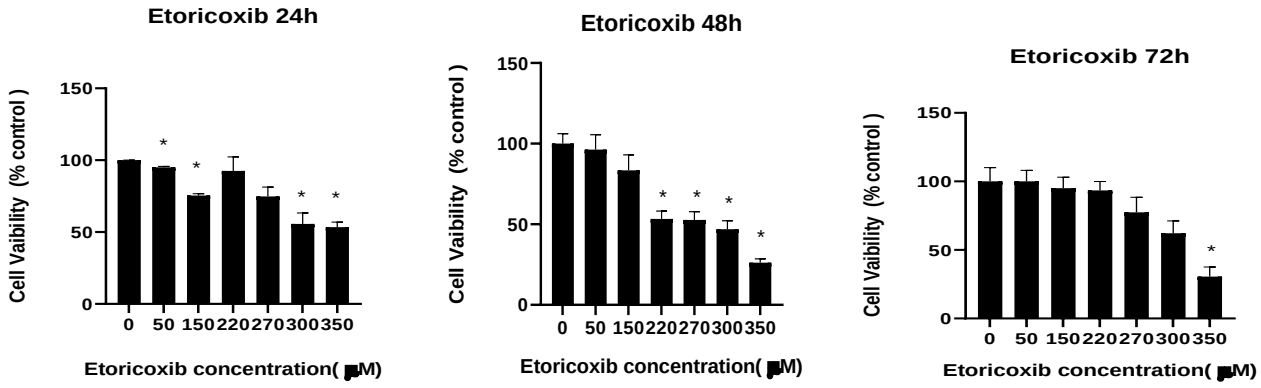


Figure 1: (continued) Effect of COX inhibitors on HepG2 cells viability.

(C) Effect of Etoricoxib on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Figure 1D:

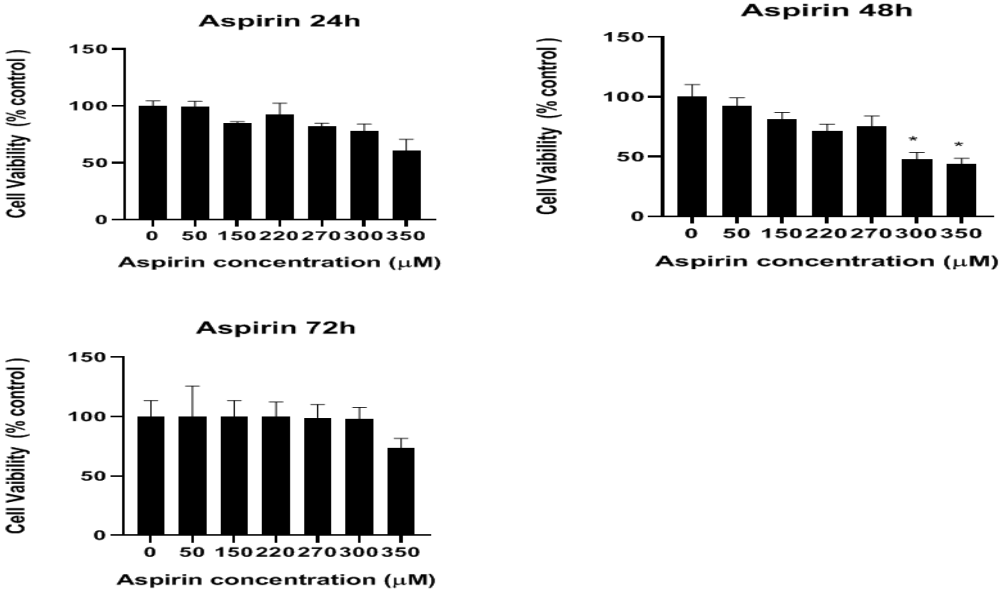


Figure 1: (continued) Effect of COX inhibitors on HepG2 cells viability.

(D) Effect of Aspirin on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Figure 2A:

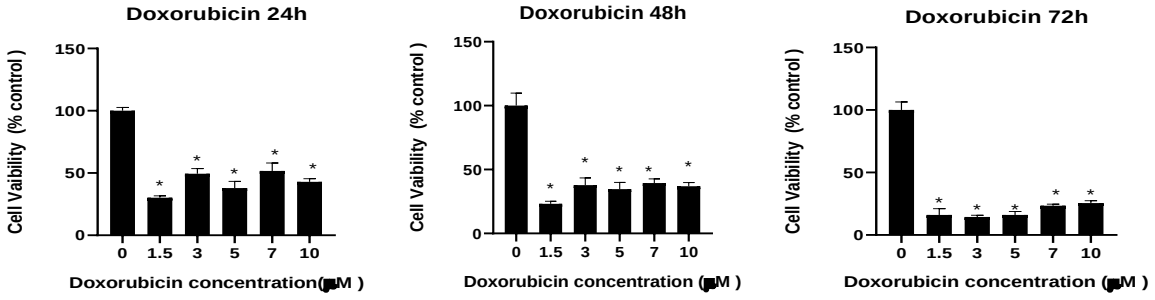


Figure 2: Effect of chemotherapeutic agents on HepG2 cells viability.

(A) Effect of Doxorubicin on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Figure 2B:

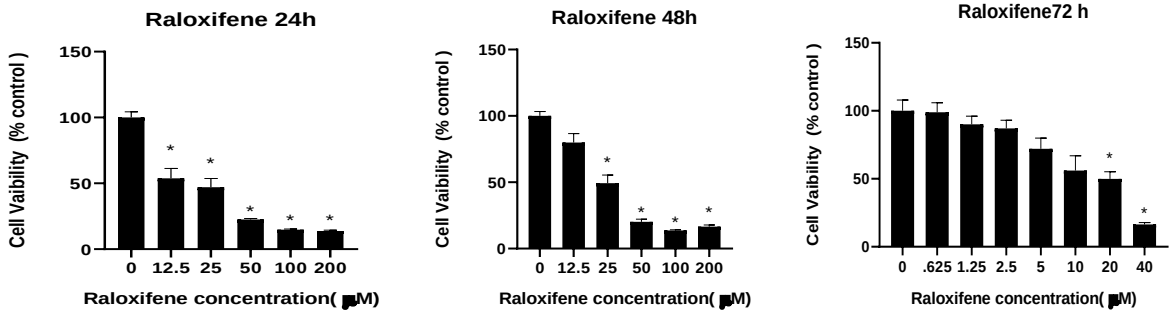


Figure 2:(continued) Effect of chemotherapeutic agents on HepG2 cells viability.

(B) Effect of Raloxifene on the viability of HepG2 cell line. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate in three independent experiments. ***** P< 0.05 is significantly different from the untreated control group.

Table 1: The 50% inhibitory concentration (IC_{50}, µM) ± SD for COX inhibitors, Doxorubicin, and Raloxifene against HepG2 cell line at different incubation periods

Treatment	24 h	48 h	72 h
Celecoxib	54±1.28	60±1.9	50±2.0
Indomethacin	378.5±3.8	308.3±6.0	349.1±4.0
Etoricoxib	398.5±8.5	260.9±7.8	310.1±7.6
Aspirin	444.4±8.0	334.5±10.0	363.6±5.6
Doxorubicin	3.9±0.1	2.4±0.08	1.45±0.05
Raloxifene	17.8±1.0	25.7±1.3	14.0±0.5

Figure 3A

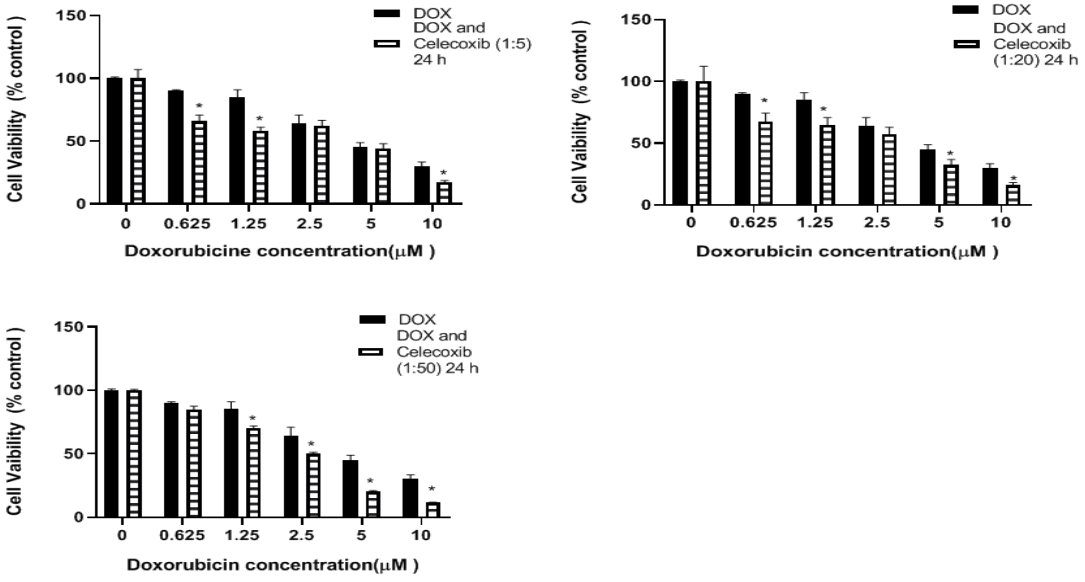


Figure 3: Effect of combination of Celecoxib and chemotherapeutic agents on the viability of HepG2 cell line. **(A)** Effect of combination between Celecoxib and Doxorubicin on HepG2 cells for 24h. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate repeated three independent times. *P< 0.05 is significantly different from the respective chemotherapeutic treatment alone.

Figure 3B

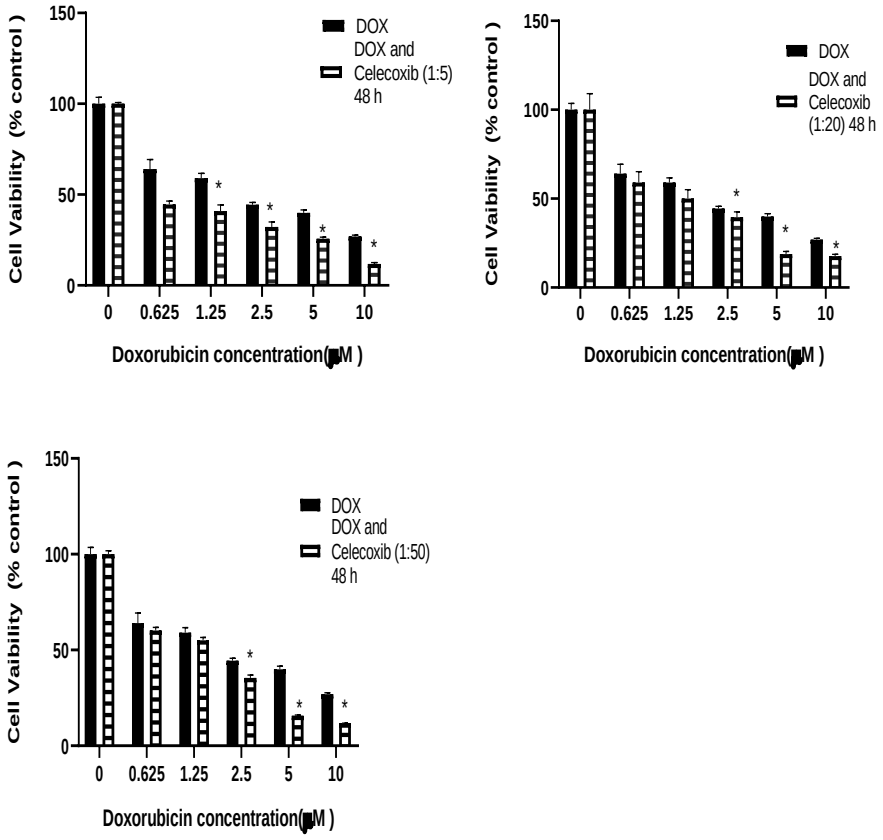


Figure 3:(continued) Effect of combination of Celecoxib and chemotherapeutic agents on the viability of HepG2 cell line. (B) Effect of combination between Celecoxib and Doxorubicin on HepG2 cells for 48h. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate repeated three independent times. *P< 0.05 is significantly different from the respective chemotherapeutic treatment alone.

Figure 3C

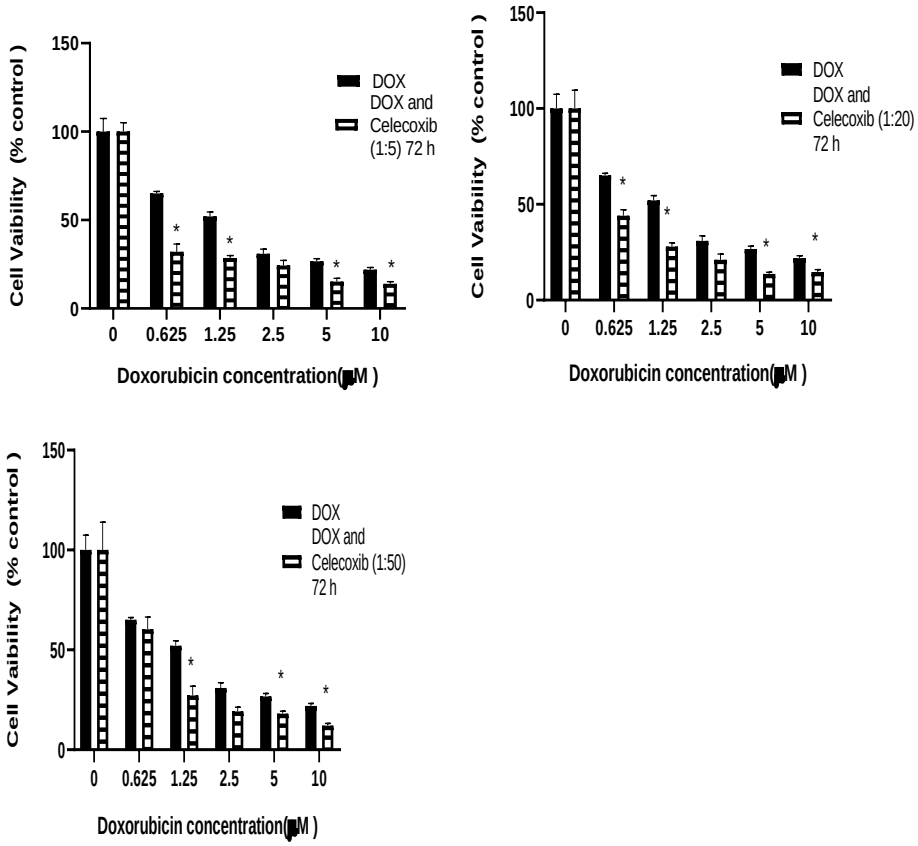


Figure 3:(continued) Effect of combination of Celecoxib and chemotherapeutic agents on the viability of HepG2 cell line. (C) Effect of combination between Celecoxib and Doxorubicin on HepG2 cells for 72h. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate repeated three independent times. *P< 0.05 is significantly different from the respective chemotherapeutic treatment alone.

Figure 3D

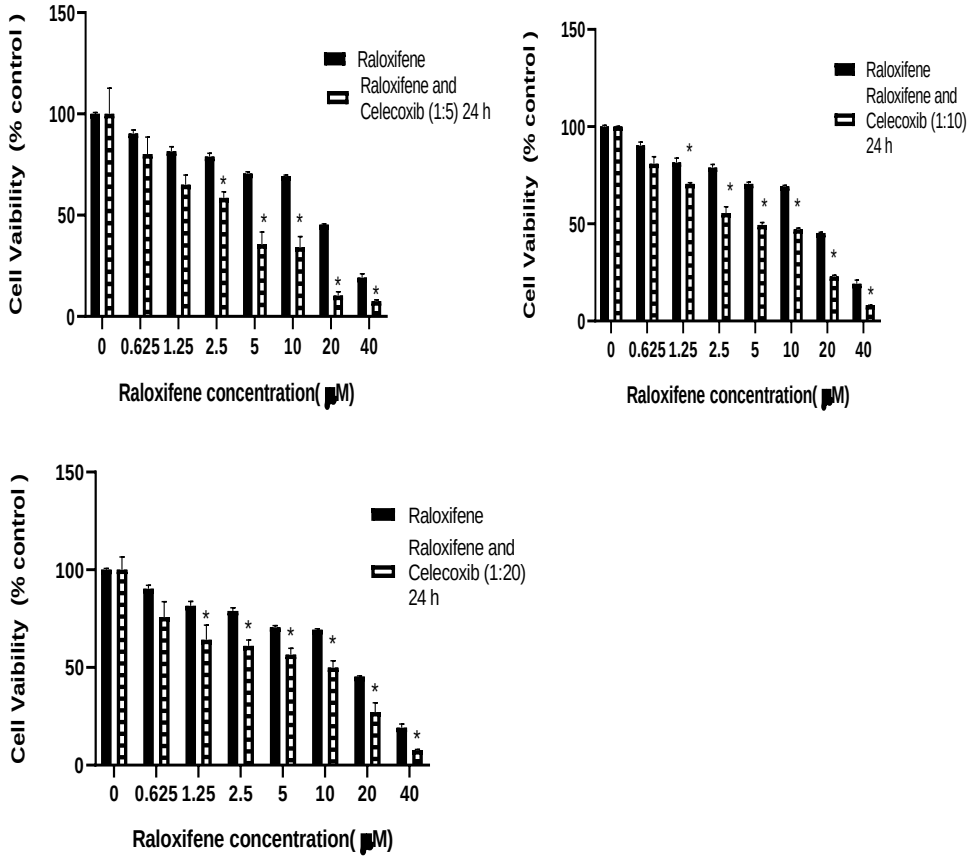


Figure 3:(continued) Effect of combination of Celecoxib and chemotherapeutic agents on the viability of HepG2 cell line. (D) Effect of combination between Celecoxib and Raloxifene on HepG2 cells for 24h. The data shown represent the mean percentages of cell viability ± SD. Each experiment was performed in duplicate repeated three independent times. *P< 0.05 is significantly different from the respective chemotherapeutic treatment alone.

Table 2: Combination Index (CI) values for combined treatment of Celecoxib and chemotherapeutic agents against HepG2 cell line.

Combination	Combination Ratio	CI 24 h	CI 48 h	CI 72 h
Doxorubicin+ Celecoxib	1:5	0.6	0.8	0.3
Doxorubicin+ Celecoxib	1:20	0.4	0.6	0.2
Doxorubicin+ Celecoxib	1:50	0.2	0.45	0.5
Raloxifene+ Celecoxib	1:5	0.3	NA	NA
Raloxifene+ Celecoxib	1:10	0.5	NA	NA
Raloxifene+ Celecoxib	1:20	0.4	NA	NA

Figure 4A:

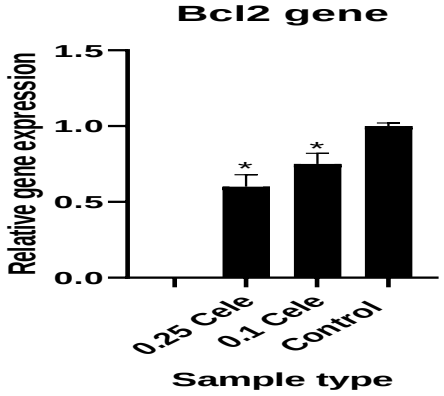


Figure 4: Change in relative gene expression with different sample types.

(A) Effect of Celecoxib on *Bcl2* gene expression in HepG2 cells. 0.25 Cele represents treatment with 0.25 IC_{50} of Celecoxib, 0.1 Cele represents treatment with 0.1 IC_{50} of Celecoxib, control represents control untreated HepG2 cells. The data shown represent the mean percentages of relative gene expression ± standard deviation (SD). Each experiment was performed in duplicate. *P< 0.05 significantly different from respective control HepG2 cells.

Figure 4B

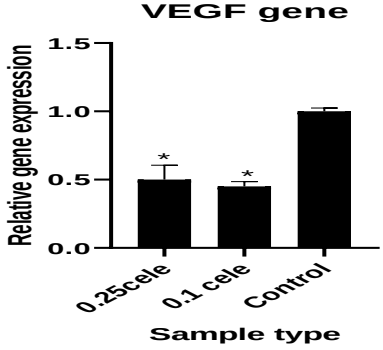
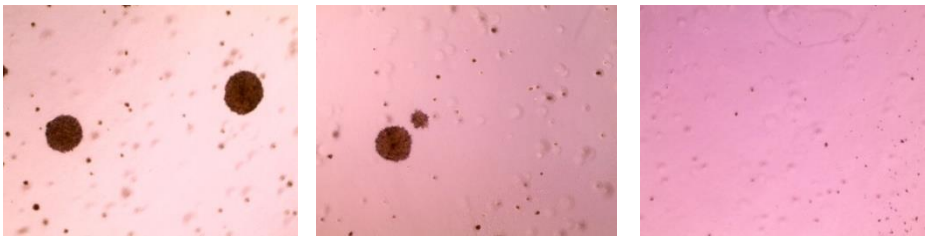


Figure 4:(continued) Change in relative gene expression with different sample types.

(B) Effect of Celecoxib on *VEGF* gene expression in HepG2 cells. 0.25 Cele represents treatment with 0.25 IC_{50} of Celecoxib, 0.1 Cele represents treatment with 0.1 IC_{50} of Celecoxib, control represents control untreated HepG2 cells. The data shown represent the mean percentages of relative gene expression ±SD. Each experiment was performed in duplicate. *P< 0.05 significantly different from respective control HepG2 cells.

Figure 5A:

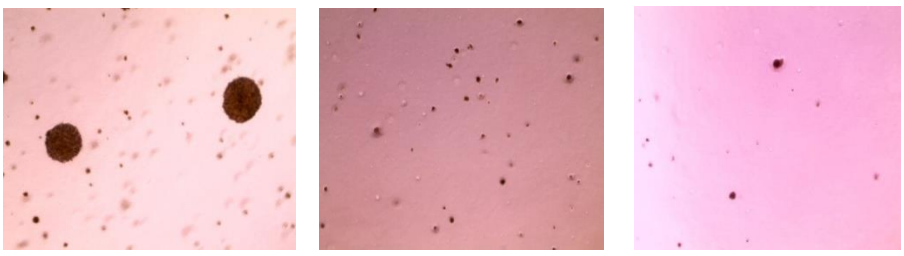


Control group **Half IC_{50} of Celecoxib** **IC_{50} of Celecoxib**

Figure 5: The effect of COX inhibitors on colony formation.

(A) Colony formation assay was performed on HepG2 cells, with control non treated group, group treated with IC_{50} of Celecoxib, and other treated with half IC_{50} of Celecoxib.

Figure 5B:



Control group **Half IC_{50} of Etoricoxib** **IC_{50} of Etoricoxib**

Figure 5: (continued) The effect of COX inhibitors on colony formation

(B) Colony formation assay was performed on HepG2 cells, with control non treated group, group treated with IC_{50} Etoricoxib, and other treated with half IC_{50} Etoricoxib.

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التحقيق في الآليات الجزيئية الكامنة وراء النشاط المضاد للتكاثر والمضاد للورم لمثبطات انزيم COX2 ضد خلايا سرطان الكبد

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ملخص

يعد سرطان الكبد أو سرطان الخلايا الكبدية أحد أكثر أنواع الأورام الخبيثة شيوعًا في جميع أنحاء العالم. على الرغم من وجود تطورات في علاج سرطان الخلايا الكبدية، إلا أن تشخيصه لا يزال ضعيفًا بسبب تكرار حدوثه وانتشاره. في هذه الدراسة ، لقد تم تقيم التأثيرات المضادة للتكاثر والمضادة للهجرة لمثبطات COX2 في خلاياHepG2 ، وهو أحد انواع خلايا سرطان الكبد. بالإضافة إلى ذلك ، قمنا بتقييم التأثيرات المشتركة لمثبطات COX2 الانتقائية (سيليكوكسيب) مع أدوية العلاج الكيميائي ، وكذلك الآلية التي تقوم بها هذه المثبطات لقتل الخلايا السرطانية. تم تقييم التأثيرات المضادة للتكاثر والتأثيرات المشتركة لمثبطات COX2 مع ادوية العلاج الكيميائي بواسطة فحص ال .(MTT)تم تقييم تأثير مثبطات COX2 على التعبير الجيني باستخدام .RT-PCR وتم تقييم اثرها على تكوين المستعمرات باستخدام فحص تشكيل المستعمرات . لقد أظهرت النتائج أن مثبطات COX2 تعمل بشكل كبير على تثبيط نمو خلايا سرطان الكبد HepG2 بطريقة تعتمد على التركيز. نتج عن العلاج المشترك لسيليكوكسيب مع دوكسوروبيسين و رالوكسيفين تأثيرًا تآزريًا. بالإضافة إلى ذلك ، قلل علاج سيليكوكسب بشكل كبير من التعبير عن جينات Bcl2 و VEGF في خلايا .HepG2 ولم يتم الكشف عن مستقبلات COX2 في جميع عينات خلايا .HepG2 كشفت نتائجنا أيضًا أن العلاج بمثبطات COX2 قلل بشكل كبير من عدد وحجم االمستعمرات التي تكونها خلايا .HepG2 . الخلاصة: قللت مثبطات COX2 من نمو خلايا HepG2 وكذلك جعلتها حساسة اكثر لدوكسوروبيسين ورالوكسيفين. وتم الكشف على أن قدرة سيليكوكسيب على التقليل من نمو الخلايا يكون من خلال تقليل التعبير الجيني لكل من جيني Bcl2 و VEGF. بينما لم يتم الكشف على وجود جين COX2 في خلايا HepG2 لذلك يبدو ان مثبطات COX2 تعمل على تقليل نمو خلايا HepG2 من خلال اليات اخرى غير تثبيط مستقبلات COX2 وايضا لوحظ انخفاض كبير في تكوين المستعمرات الناتجة عن خلايا HepG2 عند استخدام مثبطات COX2 بتركيز مكافئ لـ IC50.

الكلمات الدالة: سرطان الخلايا الكبدية، مثبطاتCOX2 ، سيليكوكسب.

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