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Aspirin and celecoxib revealed anticancer and immunomodulatory effects on colon cancer cells

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Abstract---Colorectal cancer is one of the most common malignancies in the world. Clinical evidence suggests that non-steroidal anti-inflammatory drugs (NSAIDs) were found to reduce the risk of colonic adenoma and colorectal cancer occurrence or recurrence and are more beneficial in acute inflammatory disorders than in chronic inflammatory diseases, implying that NSAIDs target the early stages of the inflammatory response. Celecoxib inhibits tumor initiation and tumor cell proliferation. Aspirin inhibits the constitutive isoform of the platelet enzyme cyclooxygenase-1 (COX-1) and the inducible isoform cyclooxygenase-2 (COX-2) which is expressed by cytokines, and some growth factors. In this study, different concentrations of both Celecoxib and Aspirin in various sets were applied to investigate their effects on colon cancer cells (SW480) proliferation and cytokines production. Our result reported a significant decrease in cells viability after the treatment with aspirin and celecoxib alone or in combination. Celecoxib significantly decreased IL-6 levels, while Aspirin treatment showed no significant change in IL-6, IL-12, and TNF- α levels. In conclusion, non-steroidal anti-inflammatory drugs (NSAIDs) have pronounced anti-proliferative effects on colorectal cancer cell lines.

Keywords---SW 480, colon cancer, Aspirin, celecoxib, NSAIDs, anti-inflammatory.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in the world and is regarded as one of the major killers (Labianca et al., 2010). This disease is linked to dysregulation and overexpression of the prostaglandin-synthesizing enzyme cyclooxygenase (COX) and overproduction of prostaglandin, abnormal cell and tissue adjustments regarding vascularization (Ettarh et al., 2021). Most colon cancers start as small benign adenomas (polyps) which may turn malignant with time. Risk factors for developing colon cancer include the hereditary condition referred to as “familial adenomatous polyposis” (FAP), a condition in which the colonic mucosa develops hundreds to thousands of polyps, and which carries a near 100% risk of turning malignant (Kallenbach-Thieltges et al., 2013). Lynch syndrome (HNPCC, Lynch syndrome) is an autosomal dominant genetic disorder. It is caused by a mutation in one of four genes in the DNA mismatch repair pathway, and it increases the risk of different cancers, including colon and endometrial cancers (Steinke et al., 2013). Familial CRC is a heterogeneous condition that includes patients with unrecognized hereditary syndromes and patients with seemingly sporadic forms (Armelaio & Pretis, 2014).

Colorectal cancer can be treated by surgery, radiation therapy, and chemotherapy (Granados-Romero et al., 2017). Non-steroidal anti-inflammatory drugs (NSAIDs) were found to reduce the risk of colonic adenoma and colorectal cancer occurrence or recurrence (Lanza et al., 2009). The COX pathway produces prostaglandins (PGs), which are the end products of fatty acid metabolism. They've long been recognized as important physiological and pathological mediators in a variety of therapeutic settings, including inflammation, pain, pyrexia, cancer, and neurological illnesses (Marshall & Blikslager, 2011). Clinical evidence suggests that NSAIDs are more beneficial in acute inflammatory disorders than in chronic inflammatory diseases, implying that NSAIDs target the early stages of the inflammatory response (Federico, 2015). Aspirin and other NSAIDs have been shown to cause apoptosis via mitochondrial routes (cytochrome c release and caspase-9 activation) and extrinsic pathways (caspase-8 activation). NSAIDs have been shown to down-regulate Bcl-2- an expression which Potential mechanisms of NSAIDs-induced apoptosis (Jana, 2008).

Aspirin is an inhibitor of the constitutive isoform of the platelet enzyme cyclooxygenase- 1 (COX-1) and the inducible isoform cyclooxygenase-2 (COX-2) which is expressed by cytokines, inflammatory stimuli, and some growth factors. The antithrombotic action of ASA is through the irreversible inactivation of COX-1 leading to the prevention of thromboxane-A2 (TXA2) biosynthesis and thus inhibits platelet aggregation(Xiao et al., 2019). While inhibiting COX-1 or COX-2 inhibits carcinogenesis in mice models, the function of COX enzymes in aspirin's anti-cancer effects is unknown, especially because daily doses of aspirin used for vascular prophylaxis are not thought to be sufficient for prolonged COX inhibition in systemic tissues. COX-2's prognostic value has not been demonstrated in other studies. (Coyle et al., 2016).

Celecoxib inhibits tumor initiation and tumor cell proliferation. According to epidemiological research, patients with the genetic family adenomatous polyposis

(FAP) condition have a reduced incidence of colonic polyps and a lower risk of colon cancer (Jendrossek, 2013).

Materials and Methods

Cell line

Sw 480 colon cancer cell line was kindly supplemented from the cancer research laboratory/college of medicine/university of Babylon. Cells were grown in RPMI (1460) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum.

Materials

Aspirin and Celecoxib were purchased from CDH, India. The stock solution of these drugs was prepared by dissolving the required weight in DMSO (Himedia, India). Working concentrations were prepared by diluting the appropriate volume of the stock solution in a complete RPMI medium according to the dilution low.

Cytotoxicity assay

Cells were seeded in 96- tissue culture plate and incubated overnight. The next day, when the confluency of the cells reaches more than 90%, the plate is ready for treatment. Before the addition of the testing material, the medium was discarded and 200 μ L of the serial dilutions were added to each well. Serial dilutions of Aspirin started from 2000 to 31 μ g/ml and from 1000 to 62 μ g/ml for Celecoxib were prepared and tested to evaluate their action on cells viability. Then, the plates were covered and incubated for 24 hours at 37C⁰. MTT assay was used to detect the cytotoxic effect of each material at the end of the incubation period.

Immunoassays

After the treatment with the drugs and incubation for 24 hours, the medium was withdrawn and frozen at -20 C until immunoassay was performed for cytokines measurement using the ELISA method.

Results

Effect of aspirin on cell viability

Our result indicated a significant ($P < 0.001$) decrease in cells viability at the concentration of (31 μ g/ml) of aspirin. Results also showed a non-significant increase in cells proliferation at concentrations of (125 and 250) μ g/ml after a period of 24 hrs in comparison with the control group as indicated in the figure (1).

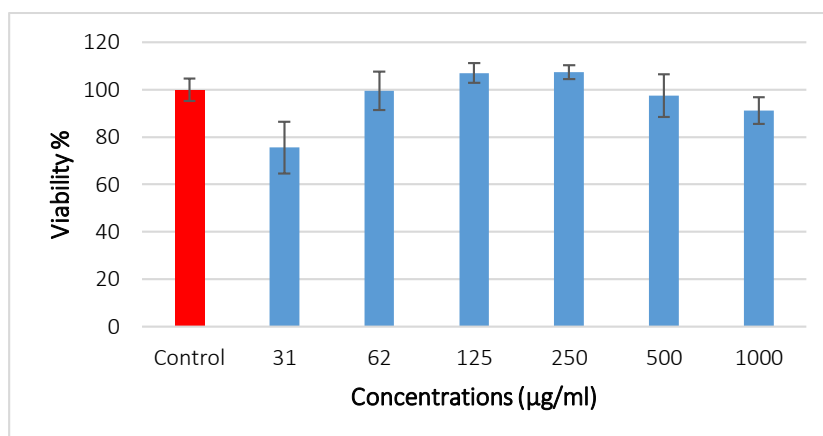


Figure (1): Effect of different concentrations of Aspirin on the viability of SW colon cancer cell line.

Effect of Celecoxib on cell viability

There was a highly significant ($P < 0.001$) decrease in cells viability for all Celecoxib concentrations used after incubation for 24hrs in comparison with the control group, as shown in figure (2).

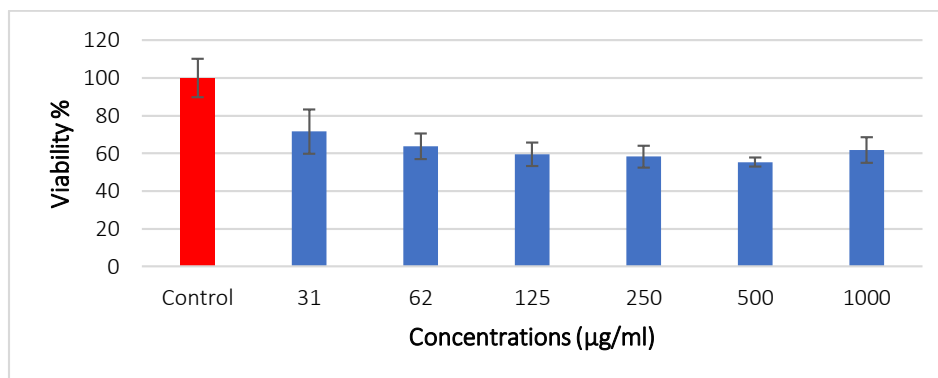


Figure (2): Effect of different concentrations of Celecoxib on the viability of SW colon cancer cell line.

Effect of (aspirin-celecoxib) combination on cell viability

In this experiment, equal concentrations of both drugs were prepared together and then were added to the cells. The result showed a highly significant ($P < 0.001$) decrease in cell viability for all concentrations used after incubation for 24hrs with the drugs in comparison with the control group, as shown in figure (3).

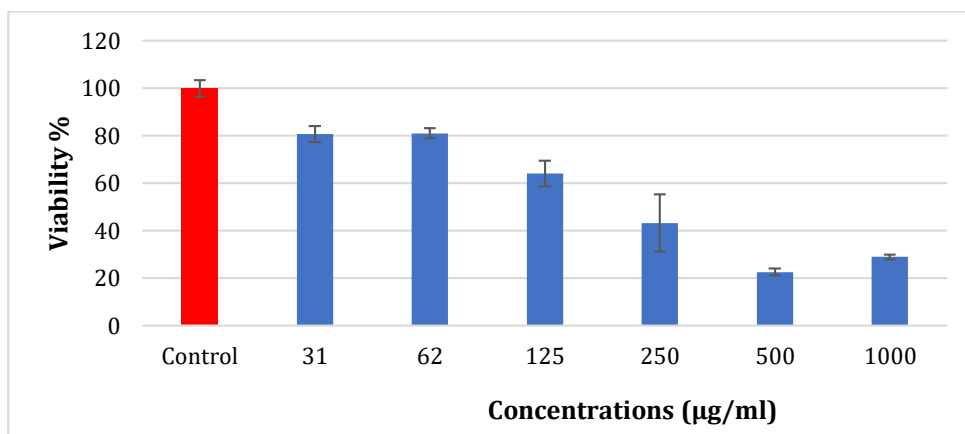


Figure (3): Effect of (Aspirin – Celecoxib) combinations at equal concentrations on the viability of SW colon cancer cell line.

Effect of Aspirin treatment on the cytokines production

The result showed that there was no statistically significant effect ($P > 0.001$) of the Aspirin treatment on the production of IL-6, IL-12, and TNF- α in comparison to the control group as shown in figures (4) A, B, and C respectively.

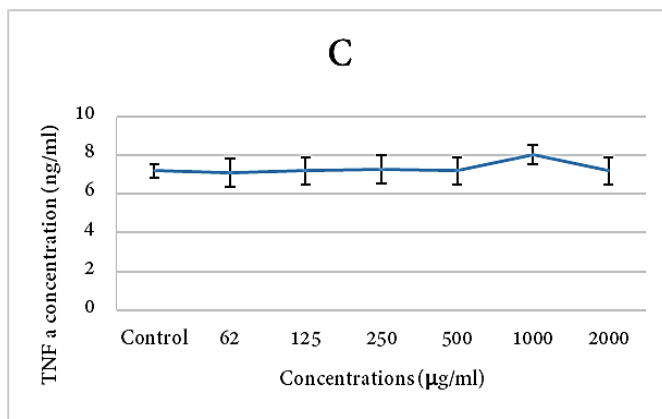
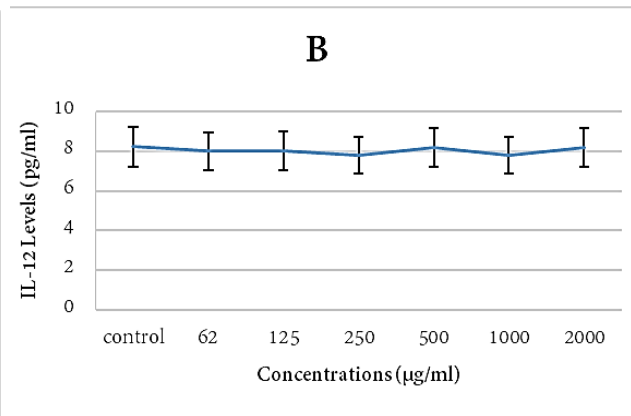
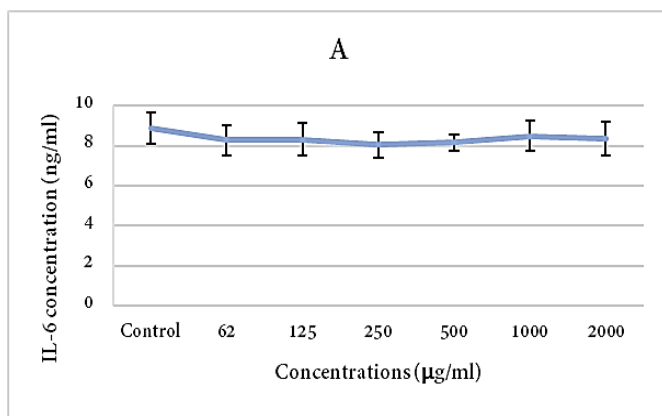


Figure 4: Effect of different concentrations of Aspirin drug on A)IL-6, B) IL-12, and C)TNF- α

Effect of Celecoxib drug on cytokine production.

The result showed that was a statistically significant ($p \leq 0.001$) decrease when using Celecoxib drug for concentration (500, 1000 $\mu\text{g/ml}$) on IL-6, while there was no statistically significant effect ($P > 0.001$) of the Celecoxib treatment on the production of IL-6 for concentration (31, 62, 125, 250 $\mu\text{g/ml}$), IL-12 and TNF- α for all concentration in compared to the control group as shown in figure (5) A, B, and C respectively.

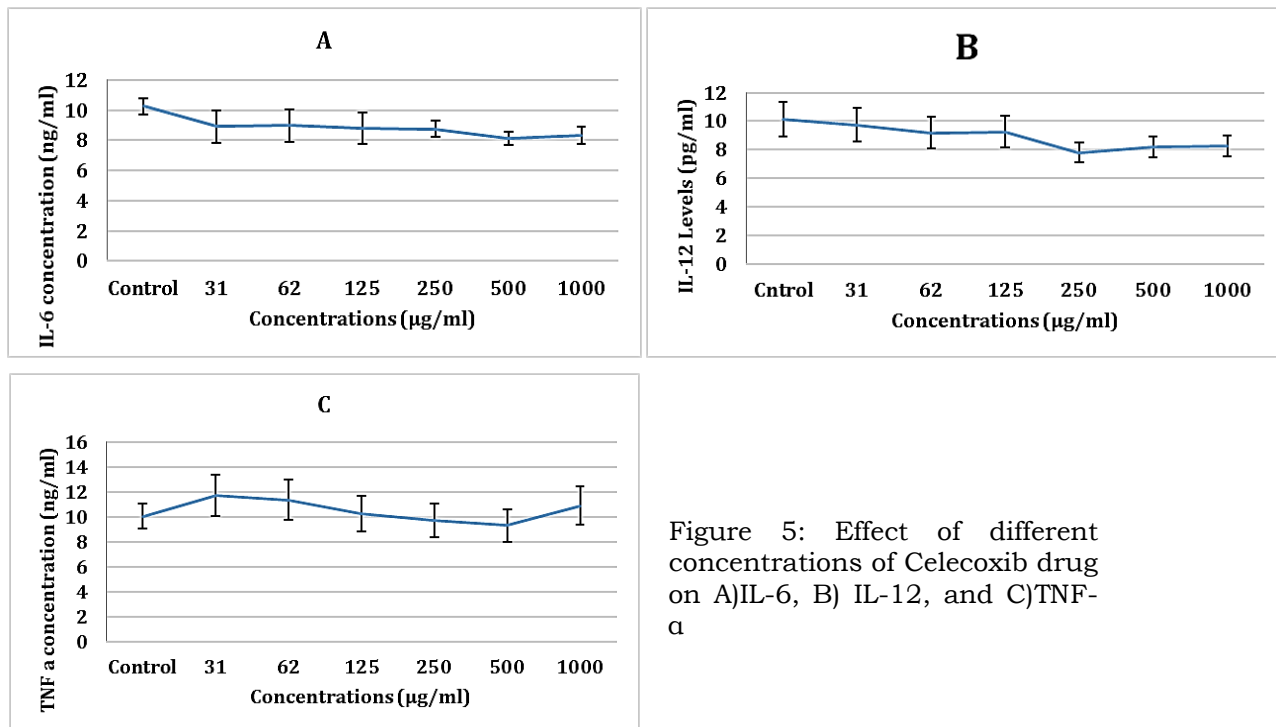


Figure 5: Effect of different concentrations of Celecoxib drug on A)IL-6, B) IL-12, and C)TNF- α

Discussion

Our result revealed that NSAIDs especially aspirin and celecoxib have a cytotoxic effect on colorectal cancer. Low quantities of aspirin mostly exert their toxicity via the necrotic route, according to a study, which also suggests that apoptosis occurs in cells exposed to aspirin and its derivatives. Apoptosis activation in a subset of cells could indicate a sensitivity linked to a specific cell cycle stage because aspirin inhibits the nuclear factor kappa B (NF- κ B) pathway (Deb et al., 2011). In colon cancer, cells line substantive synergy was observed when aspirin was combined with cisplatin and oxaliplatin with a decrease in ED50 for these platinum compounds. Given the common use of oxaliplatin in the treatment of colorectal cancer and with a clinically significant side effect of oxaliplatin being peripheral neuropathy, A reduction in its ED50 as a result of the combination with aspirin may have clinical utility: a reduction in drug concentration will result in toxicity reduction and also delay or minimize the induction of drug resistance

(Kilari et al., 2019). Most transcriptional changes were not significant but the overall trend was down-regulation, possibly reflecting the toxic effect of aspirin on the transcriptional machinery (Lai et al., 2010).

Celecoxib can induce severe oxidative stress, and mitochondrial redox homeostasis, and promotes colon cancer cell apoptosis (Z. Zhang et al., 2018). Celecoxib has appeared anti-cancer activity in many different types of cancer cells and animal models including liver cancer. Celecoxib can not only inhibit the proliferation of a set of tumours but also induce apoptosis of tumor cells. Furthermore, its inhibition of cancer cells appears to be time and concentration-dependent (Hu et al., 2020). Celecoxib and its exceptional ability to target metastatic cancer cells and synergize with chemotherapy. Synergistic anticancer effects have been attained by combining celecoxib in murine models of colorectal cancer with either 5-fluorouracil (5-FU) (Ralph et al., 2018).

When celecoxib combined with aspirin inhibited cell proliferation and induced apoptosis to a significantly greater extent than that observed after treatment with either drug alone. Celecoxib and aspirin in combination induced apoptosis of Non-small cell lung cancer (NSCLC) cells by activating caspase-8, -9, and -3. Subsequently, activation of caspases leads to PARP cleavage and nuclear condensation, which ultimately results in apoptosis. Indicate that aspirin can increase the sensitivity of (NSCLC) cells to celecoxib through the caspase-mediated apoptosis pathway and the endogenous apoptosis pathway (X. Zhang et al., 2020).

Aspirin could induce apoptosis of colorectal cancer cells by the mechanisms involving downregulation of the IL-6/signal transducer and activator of transcription 3 (STAT3) signalling pathway, therefore aspirin is an effective treatment agent for CRC by blockading IL-6-STAT3 signalling pathway (Tian et al., 2011).

Celecoxib could inhibit IL-6/IL-6R-induced STAT3 activation in hepatocellular carcinoma (HCC) cells. Celecoxib could combine with other anticancer agents to overcome the drug resistance. IL-6/STAT3 targets some genes involved in anti-apoptosis such as Bcl-2, Bcl-XL, and Survivin. Celecoxib treatment decreased the expression of these STAT3 downstream genes, which inhibited the resistance of cancer cells to anticancer drugs. The results suggest that celecoxib may be a candidate for HCC therapy by inhibiting the IL-6/STAT3 pathway and could be combined with other anticancer drugs to reduce drug resistance caused by STAT3 (Liu et al., 2011).

Conclusions

Non-steroidal anti-inflammatory drugs (NSAIDs) have anti-proliferative effects on colorectal cancer cell lines when taken alone or in combination and they may use for the protection from colon cancer.

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