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Effect of Aspirin, Sulindac and Celecoxib and Their Combination on Cell Viability and Cytokine Level in Colorectal Cancer Cell Line SW480

A thesis

Submitted to the Council of the College of Medicine, University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology & Toxicology

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بِسْمِ اللَّــهِ الرَّحْمَـٰنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

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(سورة المجادلة الآية ١١)

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We certify that this thesis entitled by (Effect of Aspirin, Celecoxib and Sulindac on Cellular Viability and Cytokine Level in SW480 Colorectal Cancer Cell Line) under our supervision at the department of pharmacology. College of medicine, university of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in pharmacology and toxicology.

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We certify that we have read this thesis entitled (Effect of Aspirin, Celecoxib and Sulindac on Cellular Viability and Cytokine Level in SW480 Colorectal Cancer Cell Line) and as an examining committee examined the student (**Rusul Kadhim Bukhet**) in its contents and that in our opinion it meets the standard of a thesis for the degree of Master, in Pharmacology and toxicology.

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Dedication

I dedicate this thesis

to my great and supportive Mother.

To my lovely husband and children

Whose love, encouragement and prays to make me able to get such success.

My wonderful sisters whose supported me..

Along with the hardworking and respected supervisors and all who's inspired me

Acknowledgment

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Summary

Colorectal cancer (CRC) is one of the most common cancers worldwide, and it ranks alongside lung, prostate, and breast cancer as one of the top killers. Colorectal adenocarcinoma is another name for colorectal cancer. Individuals with adenomas that are not removed have a higher risk of CRC, whereas polypectomy reduces the risk of CRC. The incidence, etiology, molecular mechanisms, and outcome of colorectal cancer (CRC) vary depending on the tumor's location. Treatment options include surgery, radiation, and chemotherapy, which are all accompanied with a slew of side effects, toxicity, and cost, all of which have a substantial impact on patients' quality of life. As well-known COX inhibitors, non-steroidal anti-inflammatory medications (NSAIDs) are unavoidably a prominent anticancer anti-inflammatory choice in cancer therapy and prevention. Use of NSAIDs in colorectal cancer because it cheeps and can use as prophylactic for CRC and can use in combination with other cancer drugs.

The purpose of the present study is to investigate the cytotoxic effect of non -steroidal anti-inflammatory drugs (aspirin, sulindac, and celecoxib) on the SW480 colorectal cancer cell line.

The practical work of the research study was performed at the postgraduate student’s research laboratory /department of pharmacology and toxicology/ college of the medicine/ University of Babylon.

The immunomodulatory effects of NSAIDs were detected by measuring the cytokines production in the supernatant of colorectal cancer cells in response to NSAIDs.

Experiment included: Four columns of six replicates of 96-well plates that were seeded with colon cancer SW480 cell lines in a concentration of

5*105 cells. Column No.1 with six replicates was considered as a control group, and the three subsequent columns were exposed to each drug (aspirin, sulindac and celecoxib) in serial dilutions of (1000, 500, 250, 125, 62.5,31 µg/ml), four replicate for each concentration. Incubated once for 24 hours. The effects of (aspirin, Sulindac and celecoxib) on the growth of the colon cancer line were assessed by MTT assay.

As in the above work, but exposure to a combination of NSAIDs (sulindac-aspirin, celecoxib-sulindac, aspirin –celecoxib). The effects of this combination on growth of colon cancer line were assessed by MTT assay.

The cytotoxicity results showed a significant ($P<0.05$) decrease in the viability of SW 480 for all concentrations of sulindac, celecoxib, sulindac-celecoxib, aspirin-celecoxib in comparison to the control group.

The cytotoxicity results showed a significant ($P<0.05$) decrease in the viability of SW 480 for concentration (31µg/ml) of aspirin and the concentrations (1000,500,250 µg/ml) of aspirin-sulindac in comparison to the control group.

ELISA assay was performed to detect the levels of Interleukin-6, interleukin-12, tumor necrosis factor-α. The result showed that was a statistically significant ($p<0.05$) decrease when using the Celecoxib drug for concentration (500, 1000 µg/ml) on IL-6.

Our results showed were a statistically significant increase for concentration 1000µg/ml when used sulindac drug on the production of IL-12 and on the production of TNF-α for concentration (500, 1000µg/ml) in comparison with the control group.

The result showed that there was a statistically significant ($p<0.05$) increase when used (aspirin- sulindac) combination treatment on the

production of (TNF-α) α compared to the control group. While the (celecoxib - sulindac) combinations treatment a statistically significant increase ($P<0.05$) for concentration (125,250,1000 µg/ml) on IL-12.

In Conclusion the result showed non-steroidal anti-inflammatory Drugs (NSAIDs) have anti-proliferative effects on colorectal cancer cell lines when taken alone or in combination between it and immune-modulatory effects on cytokine production, according to the findings.

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List of abbreviations

Abbreviation	Meaning
NSAIDs	Non-steroidal anti-inflammatory drugs
COX	Cyclo-oxygenase enzyme
GI	Gastrointestinal
DNA	Deoxyribonucleic
PGs	Prostaglandin
mg	Milligram
µg	Microgram
AA	Arachidonic acid
LOX	Lipoxygenase enzyme
TAX	Thromboxane
RA	Rheumatoid arthritis
CME	Cystoid macular edema
PPI	Proton pump inhibitor
NF-kB	Nuclear factor-kappa B
CRC	Colorectal caner

SS	Sulindac sulfide
WHO	World health organization
TNF-α	Tumor necrosis factor-alpha
IL-6	Interleukin—6
IL-12	Interleukin-12
IL-10	Interleukin-10
FAP	Familial adenomatous polyposis
MCR	Mutation cluster region
APC	Adenomatous polyposis coli
HNPCC	Hereditary non-polyposis colorectal cancer
PCR	Polymerase chain reaction
BMI	Body mass index
CTC	Computed tomographic colonography
FDA	Food and drug administration
EMEM	Eagle's Minimum Essential Medium
DMEM	Dulbecco's Modified Eagle's Medium
RPMS	Roswell Park Memorial Institute
IMDM	Iscove's Modified Dulbecco's Medium
DDW	deionized distilled water

ml	Milliliter
OD	Optical density
COX-1	Cyclo-oxygenase-1
COX-2	Cyclo-oxygenase-2
TOPK	T-lymphokine-activated killer cell-originated protein kinase
NHE-1	Na-H exchanger isoform 1
PK-PD	pharmacokinetic _ pharmacodynamic
STAT3	signal transducer and activator of transcription 3
SOC	Store-operated Ca2+ channel
NSCLC	Non-small cell lung cancer
HCC	Hepatocellular carcenomia
Bax	Proapoptotic gene
Bcl-2	Antiapoptotic gene
ROS	Reactive oxygen species
5-FU	5 fluorouracil
PBS	Phosphate buffer saline

Chapter one

Introduction

and

Literature Review

1.1Intruduction

Cancer is a hereditary illness caused by mutations in oncogenes and tumor suppressor genes in the nucleus. Two to eight so-called "driver gene" mutations are expected to be present in the atypical tumor, which influence the tumorigenic phenotype. The primary cause of cancer is nuclear genomic instability, which is seen in nearly all types of tumor cells. Sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, enhanced angiogenesis, and activation of invasion and metastasis are all hallmarks of cancer. In normal noncancerous stem cells, somatic mutations thought to be the cause of cancer occur at random during DNA replication. (Seyfried, 2015).

Cancer is a multifaceted illness characterized by multiple temporospatial abnormalities in cell physiology that eventually result in malignant tumors. The disease's biological goal is abnormal cell proliferation (neoplasia). The primary cause of illness and mortality in most cancer patients is tumor cell invasion of surrounding tissues and distant organs. Hanahan and Weinberg proposed that malignant cell proliferation might be caused by six key changes in cell physiology in a major review. The following six changes were described as hallmarks of nearly all cancers: 1) self-sufficiency in growth signals, 2) insensitivity to growth-inhibitory (antigrowth) signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained vascularity angiogenesis), and 6) tissue invasion and metastasis (Hainaut & Plymoth, 2012).

Cancers present differently in different patients, and tumors can vary within a given patient based on differences in the clonality of the cancer cells themselves and/or the surrounding microenvironment. Furthermore, some malignancies arise following chronic inflammatory

states, whereas others can subvert and/or co-opt an immune response as part of progression and metastasis(Hegde & Chen, 2020) .

Cancer is a major public health problem worldwide. Cancers of the female breast, colorectal, prostate, and lung are the most frequently diagnosed cancers in Europe. Lung cancer remains the leading cause of cancer incidence and mortality worldwide. cancer was classified and treated solely according to organs of origin or simplistic histomorphologic features(Zugazagoitia et al., 2016).

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women and significant international variations in the distribution of colorectal cancer have been observed. Risk factors for colorectal cancer include obesity, a diet low in fruits and vegetables, physical inactivity, and smoking, and as such it was once a disease primarily observed in longstanding developed nations whose populations typically exhibit these factors (Center et al., 2010).

Aspirin, a nonsteroidal anti-inflammatory drug (NSAID), is widely used as a painkiller, antipyretic or antiplatelet agent for more than 100 years. In addition to its classical anti-inflammatory function, epidemiological studies in several trials have demonstrated that prolonged aspirin use reduces cancer risk, particularly colorectal cancer (CRC) indicating a promising role of aspirin for cancer prevention. Aspirin consists of acetyl and salicylate moieties. While the salicylate group implicates in the anti-inflammatory and anti-cancer properties via targeting cyclin A2/CDK2, HMGB1 and NF-κB pathway the acetyl group causes the inactivation of cyclooxygenases (COXs) through acetylation of serine residues(Sun et al., 2017) .

Sulindac metabolites have provided useful drug probes to determine if COX inhibition is required for the chemopreventive properties of NSAIDs. As a sulfoxide prodrug, sulindac requires metabolism to a

sulfide that inhibits COX-1 and COX-2, which is responsible for its anti-inflammatory activity. A sulfone metabolite is also generated by oxidation of the sulfoxide, which does not inhibit COX or contribute to the anti-inflammatory activity of sulindac (Piazza et al., 2009).

The second-generation selective COX-2 inhibitor celecoxib appears in such an environment. Celecoxib is one of the commonly used NSAIDs in the clinic, mainly for the treatment of inflammatory diseases such as osteoarthritis, rheumatoid arthritis, and inflammatory musculoskeletal. Celecoxib which plays a role by selectively inhibiting COX-2. Compared with other NSAIDs, celecoxib shows lower toxicity side effects (such as the most common gastrointestinal bleeding and gastric ulcer). Early studies have shown that celecoxib can effectively reduce the incidence of colorectal cancer, especially inhibiting the development of familial adenomatous polyposis to colorectal cancer (Wen et al., 2020).

. Aim of The study

This study is designed to study:

1. The cytotoxic effect of non-steroidal anti-inflammatory drugs (aspirin, sulindac, and celecoxib) on human colorectal cancer SW480 cells line.
2. Effect of combination of (aspirin, sulindac, and celecoxib) on human colorectal cancer SW480 cells line.
3. Immunomodulatry (IL-6, IL-12, TNF-α)effect of non-steroidal anti-inflammatory drugs on colorectal cancer.

1.1.1 Cancer epidemiology

Cancer is the leading cause of death in both men and women, according to the WHO, followed by ischemic heart disease and stroke. Overall, 18.08 million new instances of cancer were recorded in 2018, with lung cancer (with 2.09 million cases), breast cancer, and prostate cancer being the three most common. Lung and prostate cancers are remain the most common cancers in men, followed by stomach cancer (0.68 million cases) and liver cancer. In women, breast cancer is by far the most frequent followed by lung cervix uteri and colon cancers. Notably, colon and rectal cancers altogether (colorectal cancer) would be the third overall most frequent cancer as well as the second most frequent malignancy in women and the third most frequent in men respectively (Mattiuzzi & Lippi, 2019).

In the United States, colorectal cancer is the third-deadliest malignancy. In 2016, an expected 134,490 new cases of colorectal cancer would be diagnosed, with 49,190 fatalities. Colorectal cancer ranks third in terms of new cases in males (8 percent of all new cancer cases), and third in terms of new cases in females (behind breast cancer and lung cancer) (8 percent of all new cancer cases).

1.1.2 Chronic Inflammation and Cancer

It was revealed that cancer begins in areas of chronic inflammation, and that tissue injury and inflammation caused by particular irritants enhanced cell multiplication. Inflammation and carcinogenesis share many biological targets and signaling pathways in apoptosis, cell proliferation, and angiogenesis. During chronic inflammation, dysregulation of these signaling pathways frequently results in abnormal expression of pro-inflammatory genes, which play a role in malignant transformation. Depending on the tumor microenvironment, some cytokines can be a double-edged sword in tumor formation. During

persistent inflammation, several of these anticancer cytokines may promote cell transformation and malignancy. Some of the cytokines implicated in inflammation and the tumor microenvironment include tumor necrosis factor (TNF-), interleukin-6 (IL- 6), transforming growth factor (TGF-), and interleukin-10 (IL-10). (Wong, 2019).

Cancer-related inflammation is considered a key characteristic of cancer, with a well-established link between chronic inflammation and tumor development. Chronic, dysregulated, persistent, and unresolved inflammation has been associated with an increased risk of malignancies, as well as the malignant progression of cancer in most types of cancer (Huakan Zhao et al., 2021)

1.2 Colon cancer

1.2.1 Introduction

Colon cancer is very common all around the world every year, over a million people contract the condition. Colon cancer is linked to dysregulation and overexpression of the prostaglandin-synthesizing enzyme cyclooxygenase (COX), as well as overproduction of prostaglandin, abnormal cell and tissue adjustments regarding vascularization, cell adhesion, apoptosis, and proliferation, according to a large body of experimental evidence. NSAIDs have been shown to affect intestinal tumor growth rates and modulate carcinogenesis through a variety of mechanisms, including inhibition of COX activity and disruption of prostaglandin homeostasis, disruption of nuclear factor kappa B (NF-kB) signaling and extracellular signal-regulated kinases (ERK/MAPK), induction of various apoptotic pathways, and effects on cell cycling. All of these mechanisms either contribute and enhance or

antagonize and counterbalance, the proliferative behavior that is observed in tumor cells (Ettarh et al., 2021).

Colon cancer, along with lung, prostate, and breast cancer, is one of the most common malignancies in the world and is regarded one of the major killers. In recent years, significant progress has been made in the treatment of this common disease: adjuvant chemotherapy has been shown to be successful, particularly in stage III patients, and surgery has been improved to obtain the greatest results with the least amount of morbidity. Several new target-oriented medications are being evaluated, and some have already demonstrated good activity/efficacy, primarily when used in conjunction with chemotherapy. (Labianca et al., 2010).

Long-term use of high doses of NSAIDs for cancer intervention was approached with certain precautions, due to COX/PGE2 inhibition is related to the possibility of gastrointestinal, renal, and cardiovascular toxicities. The recent efforts examining the discovery of novel, less toxic, non-COX inhibitory derivatives of NSAIDs support that COX inhibition may not be fully responsible for the anti-cancer activities of NSAIDs (Hongyou Zhao et al., 2021).

1.2.2 Histopathology

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. Another hereditary risk factor leads to a condition called hereditary non-polyposis colon cancer (HNPCC). Non-hereditary risk factors include smoking, obesity, and lack of physical exercise (Kallenbach-Thieltges et al., 2013).

The incidence, etiology, molecular mechanisms, and outcome of colorectal cancer (CRC) vary depending on the tumor's location. There are disparities in tumor stage at diagnosis between RCC and LCRC, in addition to variances in patient characteristics. RCC patients had a more advanced tumor stage than LCRC patients. Proximal colon tumors have a distinct symptomology, typically presenting with modest signs and symptoms such as microcytic anemia and weight loss, as contrast to rectal bleeding and bowel pattern changes, which are more obvious in LCRC .Furthermore, RCC was more commonly associated with higher TNM stages, larger tumors, increased frequency of vascular invasion, mucinous type, high grade, and invasive tumor border, whereas annular and polypoid tumors were more commonly associated with annular and polypoid tumors were more common in LCRC (Lee et al., 2015).

1.2.3 The Adenoma-Carcinoma Sequence

Polyps are isolated lesions that protrude above the surrounding mucosa and are frequently referred to as benign GI tumors. Most colorectal polyps in humans are hyperplastic, especially tiny polyps smaller than 5 mm in size. The majority of evidence suggests that hyperplastic polyps are not a major precursor to CRC; rather, the adenomatous polyp, or adenoma, is the most common precursor lesion. Individuals with adenomas that are not removed have a higher chance of CRC, but polypectomy reduces the risk of CRC. Adenomatous polyps frequently contain carcinoma foci, while CRC specimens frequently have residual adenomatous epithelial areas. By the third to fifth decade, people with disorders that highly predispose to adenomas, such as FAP, inevitably get CRCs. In addition to patients whose adenomatous polyps are a risk lesion for colorectal cancer (CRC), individuals with long-standing and severe ulcerative colitis have a 10-fold or greater increase in colorectal cancer (CRC)

risk compared with the general population, and dysplasia and flat adenomatous plaques may be important precursor lesions (Fearon, 2011).

1.2.4 Classification

1.2.4.1 Familial Adenomatous Polyposis

FAP is an autosomal dominant disorder in which hundreds to thousands of adenomas form in the rectum and colon during the second decade of life. If colorectal cancer (CRC) is not detected and treated early, almost all people will develop it. Until the adenomas are large and numerous enough to induce rectal bleeding or even anemia, symptoms are rare in children and adolescents. Changes in bowel habits, constipation, diarrhea, abdominal aches or palpable abdominal masses, or weight loss in young children can all lead to a recto-sigmoid examination and the discovery of polyps that are suggestive with FAP. Mutational analysis of the *APC* gene indicates that the majority of germline mutations found in patients with FAP are nonsense mutations, leading to the formation of a truncated protein (Half et al., 2009).

The role of *APC* mutations in the pathogenesis of Colorectal Cancer:

The APC gene is situated on chromosome 5q21-22 and is made up of 16 exons that code for a 2861-amino-acid long multifunctional scaffolding protein with several functional domains. APC, in combination with axin, forms a degradation site for -catenin, which it then recruits for GSK3 phosphorylation on serine-threonine. Both APC alleles are inactivated in most colorectal malignancies. Furthermore, β-catenin is phosphorylated in the degradation complex in CRC cells. Five of the 20 amino acid region repeats essential for catenin breakdown near codon 1500 were absent in several APC mutants of SW480 CRC cell lines. The

majority of oncogenic mutations in the APC gene are identified here. (Aghabozorgi et al., 2020).

APC Protein Function in the β-Catenin-Dependent Wnt Signaling Pathway

Wnt proteins are cysteine-rich glycoproteins that act as ligands for up to fifteen receptors and co-receptors in humans. Extracellular Wnt can activate a variety of intracellular signaling pathways, including the Wnt/beta-catenin dependent or canonical pathway, as well as the betacatenin-independent or noncanonical pathway. The Wnt/Ca 2+ pathway and the Planar Cell Polarity pathway are two examples of beta-cateninindependent pathways (PCP). The binding of the Wnt ligand to the LRP5/6 receptors (low-density lipoprotein receptor) and Frizzled receptors activates the beta-catenin-dependent signaling cascade. This activates Disheveled (DVL), causing the complex (Axin, GSK-3 beta, CK1, APC) to be recruited to the receptor. Without Wnt, GSK-3 beta and CK1 and subsequent sequestration in the beta-catenin destruction complex, (APC,
GSK-3 beta, CK1, Axin). (Krishnamurthy & Kurzrock, 2018)

1.2.4.2 The Hereditary Non-Polyposis Colorectal cancer (HNPCC)

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. Single colorectal adenomas or carcinomas that cannot be clinically separated from sporadic malignancies are commonly involved in the HNPCC syndrome. Colorectal cancer is common in HNPCC patients before they reach the age of 50 (average age at commencement of disease: 45 years), and about

one-third of individuals develop another HNPCC-typical tumor within ten years. (Steinke et al., 2013).

HNPCC is a heterogeneous syndrome defined by a genetic predisposition to CRC and extracolonic cancers such as those of the endometrium, stomach, ovaries, urinary bladder, small intestine, pancreas, and biliary tract. HNPCC is caused by germline mutations of any one of four major mismatch repair (MMR) genes, namely MLH1, MSH2, MSH6, or PMS2In a patient with a clinical suspicion of HNPCC, genetic testing is frequently preceded by a polymerase chain reaction (PCR) or immunohistochemistry (IHC)–based estimation of mismatch repair protein expression to assess microsatellite instability. Gene testing is recommended for patients with verified unstable microsatellite status to re-confirm the diagnosis. Following the discovery of a disease-causing mutation, relevant genetic counseling and testing for other family members is provided (Bhai et al., 2020).

1.2.4.3 Familial colorectal cancer

The development of genome-wide massively parallel sequencing, i.e. whole-genome and whole-exome sequencing, and copy number approaches had raised high expectations for the identification of novel hereditary colorectal cancer genes. Family history of cancer is one of the strongest predictors of CRC risk, being this risk higher with an increasing number of affected relatives and when CRC occurs at a young age. Crude estimates indicate that 20-25% of all CRC patients have at least one relative affected with the disease, which may be explained by shared genetic and/or environmental factors (Valle, 2016).

Non-syndromic or familial CRC is generally defined as the clustering of CRC that is distinguished from the hereditary syndromes. Familial CRC is a heterogeneous condition that includes patients with

unrecognized hereditary syndromes and patients with seemingly sporadic forms that aggregate in families. Colonoscopic surveillance is already offered to people with moderate risk due to family history (FH) of CRC, but evidence supporting reduced mortality is lacking. The heterogeneous nature of non-syndromic CRC suggests that the variation in genetic risk is likely to be a consequence of the co-inheritance of multiple low penetrance variants, some of which are common. (Armelao & Pretis, 2014).

1.2.5 Symptoms

1. A change in bowel habits that lasts more than a few days, such as diarrhea, constipation, or stool narrowing.
2. A need for a bowel movement that isn't alleviated by having one.
3. Rectal hemorrhage characterized by vivid red blood
4. Blood in the stool, which can turn it a dark brown or black color.
5. Abdominal (belly) pain or cramping
6. Weakness and exhaustion.
7. Weight loss that was not expected (Polyps et al., n.d.)

1.2.6 Risk Factor of Colorectal Cancer

The main environmental factors associated with an increased or reduced risk for colorectal malignancies and the main recommendations for primary prevention of these tumors are:

1.2.6.1 Diet

When compared to diets in poor nations, Western countries' diets are characterized by larger quantities of processed foods, red meat, and lower dietary fiber. Fiber, antioxidant vitamins, folic acid, flavones, and other micronutrients may have a preventive impact against colorectal

cancer. According to several observational studies, diets poor in fruits and vegetables are linked to an increased risk of colorectal cancer.

When the RR of colorectal cancer is evaluated across groups with the highest and lowest vegetable intake, it is roughly 0.5. In a large prospective study on the impact of fruit and vegetable eating on the development of colorectal cancer, a one-serving increase in fruit and vegetable consumption per day was linked to a covariate-adjusted RR of 1.02 A higher intake of fiber has been associated with a reduced risk of colorectal cancer in some studies (Watson & Collins, 2011).

1.2.6.2 Physical Activity, obesity and energy imbalance

Individuals of both sexes in sedentary occupations have a higher risk of colonic tumors, but the risk reduces over time for employment involving more physical activity; however, no link has been shown between physical activity and rectum cancer. The link between physical activity and the risk of colonic neoplasia is reasonably well established. Several clinical research have suggested that obese people are more likely to develop colorectal cancer; however, other studies have failed to find a link between BMI and the risk of colorectal cancer. Furthermore, the higher risk was only discovered in males, whereas the data in females was less consistent. It has been proposed that high-intensity physical activity is linked to a lean body mass might induce a metabolic
"environment" that is less amenable to cancer development, especially for tumors of the large bowel and breast (Ponz de Leon & Roncucci, 2000)

1.2.6.3 Alcohol

Despite some inconsistencies, the majority of research show a link between alcohol consumption and the development of large bowel cancer, particularly cancers of the distal colorectum. In most ecological prospective cohort and population-based case-control studies, alcohol

consumption is linked to cancer of the distal colorectum. Furthermore, alcohol consumption is linked to an increased incidence of colorectal adenoma. Acetaldehyde, a byproduct of alcohol breakdown, may inactivate N5-methyltetrahydrofolate, a type of folate necessary for methionine synthesis, inhibiting the methionine synthetase complex. Alcohol use was directly linked to the risk of colon adenoma in the Health Professionals Follow-Up Study. (Giovannucci & Willett, 1994).

1.2.6.4 Diabetes and colorectal cancer

These analyses included a total of 15 cohort studies providing data on 13,637 people with colorectal cancer. North America and Europe made up the majority of the research populations, with the Asia-Pacific region accounting for the remaining three studies. According to the pooled estimate, those with diabetes have a 20% higher risk of colorectal cancer than people who aren't diabetic. There was no evidence of heterogeneity between trials and there was no significant difference in the estimates for colon and rectal cancer.
(Huxley et al., 2009)

The proposed hypothesis is based on the premise that exposure to elevated blood-insulin levels promotes the growth of colon tumors. Growth factors are widely believed to play a central role in carcinogenesis. While it remains unproven that insulin stimulates the growth of colon tumors in humans, several lines of evidence support this scenario. First, insulin is an important growth factor for colonic mucosal cells and is a mitogen of colonic carcinoma cells *in vitro.* stimulation of IGF-1 receptors by IGF-1 orIGF-2 may increase the risk of colon cancer in humans. (Giovannucci, 1995).

1.2.6.5 Inflammatory bowel diseases

Hyperinsulinemia raises the risk of colorectal cancer by encouraging colon cell proliferation and decreasing apoptosis. There is

also a relationship between ulcerative colitis and colorectal carcinogenesis in terms of gastrointestinal inflammation and colorectal cancer, according to study. According to research, ulcerative colitis is responsible for about 1% of all colon cancer occurrences, and the risk of developing cancer is directly related to the length of time a patient has had the inflammatory condition. Crohn's disease, which causes inflammation in the intestine, has also been related to a higher risk of colorectal cancer. Additionally, certain methods of cooking meat may also increase the risk of developing colorectal cancer (Yoshihara et al., 2007).

1.2.7 Screening test of colorectal cancer

The ideal screening study should be efficient with high sensitivity and specificity, safe, available, convenient, and cheap. Current CRC screening methods are divided into invasive and non-invasive tests. The non-invasive tests include stool and blood-based tests and radiologic tests.

***The stool-based tests** currently available are the guaiac-based fecal occult blood test (gFOBT), fecal immunochemical test (FIT), and the newer fecal DNA testing (Multitarget stool DNA, MT-cDNA)

***The radiologic examinations** include double-contrast barium enema, capsule endoscopy, and computed tomographic colonography (CTC).

***Invasive tests** include flexible sigmoidoscopy (FS) and colonoscopy which offer direct visualization and detection of a colonic polyp or advanced neoplasia with the advantage of getting a pathology specimen (Issa & NouredDine, 2017)**.** 1.2.8 Colorectal Cancer Stages

1.2.8 Colorectal Cancer Stages

Stage 0

The cancer is in its earliest stage. This stage is also known as carcinoma situ or intramucosal carcinoma.

Stage I

The cancer has spread from the muscularis mucosa to the submucosa, and it may have also spread to the muscularis propria. It hasn't migrated to neighbouring lymph nodes or to any other distant locations.

Stage II A

Cancer has spread to the colon's or rectum's outer layers but has not penetrated them. It hasn't gotten to any nearby organs. It hasn't spread to any adjacent lymph nodes or to any distant locations.

Stage IIB

Cancer has grown through the wall of the colon or rectum but has not grown into other nearby tissues or organs. It has not yet spread to nearby lymph nodes or distant sites.

Stage III

Cancer has grown through the mucosa into the submucosa. It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites.

Stage IV

Cancer may or may not have grown through the wall of the colon or rectum. It might or might not have spread to nearby lymph nodes (Polyps et al., n.d.)

1.2.9 Prevention and control

1. **FOOD:** having diet of fish, fiber, vitamin D, and calcium, as well as regular exercise and aspirin use, can all help prevent the development of colorectal cancer. Dietary change combined with the intake of anti-inflammatory drugs can not only prevent the growth of new polyps but also slow the growth of existing polyps. (Yoshihara et al., 2007).

2. family/community interventions

It may also beneficial in prevention of prevent colorectal cancer. Family-based support for a family member with colorectal cancer, through the use of motivation and encouragement, when performing prevention measures such as screening, exercising, and eating a healthy diet, can be powerful learning tool to help with carcinogenesis prevention. Screening, genetic testing, and the other aforementioned environmental preventative strategies, as well as family-based interventions like these, can all play an important role in the prevention and control of disease (Yoshihara et al., 2007).

1.2.10 Treatment

Treatment for colorectal cancer is determined by a number of criteria, including the patient's overall health, the tumor's size, and its location. **1.Surgery:** most common treatment option, and the type of surgery utilized, again, depends on variables such as the location of cancer and the existence and amount of metastasis if cancer is found only in a single polyp, it can be removed during a colonoscopy. However, if cancer has affected a greater portion, then a bowel resection may be necessary (Yoshihara et al., 2007)**.**

Surgical risk stratification still one of the most important aspects of management in older patients. Age is associated with increased mortality following elective colorectal resection, up to 15.6% in patients > 80 years of age. Elderly patients with higher levels of comorbidity might be expected to have significantly greater rates of complications, longer hospital stays, and higher mortality (Millan et al., 2015).

2. Radiation Therapy: is Indicated in cases where the surgical resection margin is compromised, such as in the case of adhesion or infiltration to nearby organs or the retroperitoneal area, and is frequently used in

tandem with chemotherapy for stage III rectal cancer patients. (Granados-Romero et al., 2017)

3. **Chemotherapy:** chemotherapy for 6 to 8 months following surgery improve symptoms and prolonging survival in people with stage IV cancer primarily. The 5-Fluoracil continues to be the cytostatic mostly used in the treatment of colon cancer, those who make use of it or at least approach the fulfillment of the therapeutic standard, achieve better percentages of survival (Granados-Romero et al., 2017).

The 3 chemotherapy agents utilized to treat patients with early stage colon cancer are 5-fluorouracil (5FU), capecitabine and oxaliplatin .5-FU exerts its antitumor effects mainly through the inhibition of thymidylate synthase (TS) leading to disrupting the intracellular deoxynucleotide pools required for DNA replication. Other possible sites of action comprise incorporation into RNA with the subsequent disruption of RNA synthesis following its anabolism and incorporation into DNA resulting in its fragmentation (Vodenkova et al., 2019) .

*Adjuvant Therapy for Stage II Colon Cancer : Patients with stage II colon cancer in the MOSAIC trial had 6-year overall survival of 87% in both treatment arms, thus did not benefit from the addition of oxaliplatin to 5FU.

*High-Risk Features in Stage II Colon Cancer:5FU or 5FU/oxaliplatin chemotherapy may be offered if the following high-risk tumor features are identified in the pathology report: T4 stage, bowel perforation, bowel obstruction, poorly differentiated histology , lymphovascular invasion, perineural invasion, less than 12 lymph nodes examined, close or positive surgical margins.

* Adjuvant Therapy for Stage III Colon Cancer: For stage III colon cancer, the risk of recurrence after surgery is 50% to 60%, and adjuvant 5FU/oxaliplatin chemotherapy can reduce the risk of death by 20% (Wu, 2018).

***** In metastatic disease, agents targeting angiogenesis such as bevacizumab, ramucirumab, and aflibercept or epidermal growth factor receptor (EGFR)-directed treatments including panitumumab and cetuximab are commonly used along with FPs-based chemotherapy. Regorafenib, an angiogenesis-targeting tyrosine kinase inhibitor, or trifluridin-tipiracil (a fluoropyrimidine) are modestly active in late-stage metastatic colorectal cancer (mCRC) (Vodenkova et al., 2019).

The role of an FDA-approved antibody variant named Bevacizumab in reducing vascular endothelial growth factor (VEGF), the key angiogenesis regulator, produced by normal and neoplastic cells 50 is currently being investigated. In preclinical trials, a human monoclonal antibody against VEGF inhibited the growth of human tumor xenografts (Granados-Romero et al., 2017)**.**

Ramucirumab is a monoclonal antibody that targets VEGR-2, and in the phase III raise trial, it was shown to improve OS when patients were treated with folfiri/ramucirumab versus folfiri alone, 13.3 months versus 11.7 months (Wu, 2018).

1. 3Cell culture

1.3.1 Definition

The technique of taking cells from an animal or plant and growing them in an artificially controlled environment is known as cell culture.Disaggregation employing enzymatic or mechanical procedures

can be used to separate the cells from the tissues. Cells are occasionally produced from an existing cell line or cell strain (Bhatia, 2019).

1.3.2 History of cell culture

The use of primary monkey kidney cells in the creation of the polio vaccine animal tissue culture is the process of growing animal cells, tissue, or organs in a controlled artificial environment. Animal tissue culture was first recognized as important during the creation of the polio vaccine, which used primary monkey kidney cells (the polio vaccine was the first commercial product generated using mammalian cell cultures) (Bhatia, 2019).

1.3.3 Type of cell culture

1. Primary cultures

Primary cells are cells that have been cultivated directly from tissue.

A primary culture can be made by allowing cells to migrate out of the tissue following sterile dissection and adhere to a substrate, or by manually disaggregating the tissue with enzymes to form a cell suspension. Despite the fact that many cells cannot attach and survive in vitro, these cells are more representative of the cell types found in the tissue from which they were taken. Most primary cultures, with the exception of those produced from tumors, are varied, have a low growth fraction, and have a short lifespan. To generate a secondary culture, attached cells are trypsinized and reseeded in a fresh flask (Otero et al., 2012).

2. Cell Line

When a primary culture is subcultured or passaged it. A secondary culture is formed. Acell line that experiences indefinite growth of cells during subsequent subculturing is called a continuous cell line, whereas

finite cell lines experience the death of cells after several subcultures. Cell lines are generally immortalized or transformed cells that have lost control over division, as a result of mutations or genetic change, or as a result of primary cell was transfected with some genes that immortalized the cells. Most cell lines are tumorigenic as they originated from malignancy (Bhatia, 2019)**.**

3. Cell Strain

Cell lines are a permanently established cell culture that will proliferate forever if a proper fresh medium is provided regularly, whereas cell strains have been adapted to culture but, unlike cell lines, have a finite division potential. A cell strain is can be derived either from a primary culture or a cell line. This is done by selection or cloning of those particular cells having specific properties or characteristics (e.g. specific function or karyotype) which must be defined. (Bhatia, 2019)

1.3.4 Applications of cell culture:

1. Pharmacological uses and drug discovery

3D cultures have the potential to significantly improve cell-based drug screening and uncover harmful and ineffective compounds much sooner in the drug development pipeline than animal or clinical trials. Furthermore, they have the potential to eliminate ethically contentious animal testing.

2. **Cancer Biology Applications and Tumor Models**

Biomarkers, invasion, metastasis, and tumor angiogenesis have all been studied extensively in cancer research.

3. Applications for Gene and Protein Expression

When we compare the gene and protein expressions of cells in 3D cultures to those expressed in genuine in vivo tumors, we find a comparable result.

4. **Applications in Cell Physiology**

Different cell functions such as proliferation, adhesion, viability, morphology, microenvironment, and medication response are better understood using 3D cell cultures. (Article, n.d.)

1.3.5 Advantage of cell culture

The physiochemical environment can be controlled in cell culture (pH, temperature, osmotic pressure, and O 2 and CO 2 tension). This has a number of advantages, including the ease with which cytology and immunostaining can be performed, the ease with which quantitation can be performed, and the ability to perform studies with smaller volumes, lowering expenses. With direct access to the cell, cultures can be exposed to a reagent at a low and defined concentration. Tissue samples are normally heterogeneous; however, cultured cell lines take on a uniform appearance after one or two passages, because the cells are randomly mixed at each transfer and the selective pressure of the culture conditions tends to produce a homogeneous culture of the most vigorous cell type. As a result, experimental replicates might be quite comparable, lowering costs. reducing statistical variance (Otero et al., 2012)**.**

1.3.6 Types of cell culture media

1. **Eagle's Minimum Essential Medium (EMEM):**

Harry Eagle created EMEM from a simpler basal medium. It was one of the earliest extensively used media (BME). EMEM is made up of sodium pyruvate, a balanced salt solution, and nonessential amino acids.It's made with a lower sodium bicarbonate concentration (1500 mg/l) to work with CO2 levels of 5%.

2. Dulbecco's Modified Eagle's Medium (DMEM):

DMEM contain nearly twice many of amino acids and four times the amount of vitamins as EMEM, as well as ferric nitrate, sodium pyruvate, and certain additional amino acids. The original formulation which include 1,000 mg/L of glucose and was first reported for culturing embryonic mouse cells. A further variation with 4500 mg/L of glucose has been proved to be optimal for a culture of various types of cells. DMEM is a basal medium and contains no proteins or growth-promoting agents. As a result it requires supplementation is required to make it a full medium. It is most commonly supplemented with 5-10% Fetal Bovine Serum (FBS). DMEM use a sodium bicarbonate buffer system (3.7 g/L) and therefore requires artificial levels of CO2 to maintain the required pH.

3. Roswell Park Memorial Institute (RPMI-1640):

RPMI-1640 is a general-purpose media for mammalian cells, particularly hematopoietic cells, with a wide range of applications. The Roswell Park Memorial Institute (RPMI) in Buffalo, New York, created RPMI-1640. RPMI-1640 is a modified version of McCoy's 5A that was created for the long-term culture of lymphocytes from the peripheral blood. The bicarbonate buffering method in RPMI-1640 distinguishes it from most mammalian cell culture mediums in its normal pH 8 composition.

4. Iscove's Modified Dulbecco's Medium (IMDM):

IMDM is a highly enriched synthetic media that is ideal for high density cell cultures that proliferate quickly. IMDM is a seleniumfortified version of DMEM that contains more amino acids, vitamins, and inorganic salts than DMEM (States, 2017)

1.4 Cell model

1.4.1 SW480 Colon cancer cell line

One of the most fatal and prevalent cancerous world-wide is the colorectal cancer, despite the great advance in the medical field and chemotherapeutic agents, the drug resistance remains the unresolved problem in cancer. Therefore, establishing an effective compound with lowest side effects to fight cancer is of central priority. Herbal products have been traditionally used to prevent and treat a variety of diseases (Shanehbandi et al., 2019).

The colonic cancer SW480 cell line originates from primary tumor of an adenocarcinoma of the colon in a 50 year old male (Siekmann et al., 2019).

1.5 Cytokines

Cytokines are regulatory proteins, that are secreted by various cells, which control immune response, hematopoiesis, inflammation, wound repair and tissue morphogenic. Cytokines may be secreted, or membrane bound. The secreted cytokines may act at the same location in which they are produced as autocrine or paracrine, or over some distance as would a hormone. The cytokines that bound to the cell membrane act by cell to cell contact, allowing transfer of information from one side to another and often bidirectionally. (Walter M.Lewko and Robert K.OLdham, 2009).

1.5.1Tumor necrosis factor alpha (TNF-α)

is a multifunctional cytokine involved in apoptosis, cell survival, inflammation, and immunity acting via two receptors . Currently it is

used in cancer treatment in the isolated limb perfusion (ILP) setting for soft tissue sarcoma (STS), irresectable tumors of various histological types, and melanoma intransit metastases confined to the limb TNF-α is a 17-kDa protein consisting of 157 amino acids that is a homotrimer in solution. In humans, the gene is mapped to chromosome 6. Its bioactivity is mainly
regulated by soluble TNF-α–binding receptors. TNF-α is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells (van Horssen et al., 2006).

1.5.2 Interleukin6

IL-6 is a multifunctional cytokine that was originally characterized as a regulator of immune and inflammatory responses. Elevated expression of IL-6 has been detected in the serum and tumor tissue of patients with certain carcinomas. Abnormally high levels of IL-6 have also been reported in the serum and tumor tissue of patients with colorectal cancer (CRC). Furthermore, higher IL-6 levels are associated with increasing tumor stages and tumor size, with metastasis and decreased survival .IL-6 binds to a heterodimeric receptor, which contains the ligand-binding IL6a chain and the common cytokine receptor signal-transducing subunit gp130 (Yu et al., 2012).

1.5.3Interleukin12

IL-12 is a pro-inflammatory cytokine which induces The cells differentiation into The cells .IL-12 is composed by an heterodimeric structure of 70 kDa, which includes two covalently linked subunits, namely p40 (IL-12B) and p35 (IL-12A). The expression of the p35 and p40 genes is independently regulated. Indeed, p35 subunit is tightly regulated and requires the expression of p40 for the secretion of the biologically active cytokines. IL-12 can activate natural killer (NK) cells,

generate lymphokine-activated killer (LAK) cells, and it is involved in the generation of cytotoxic T lymphocytes. IFN-c induced by IL-12 is essential for the development of different autoimmune diseases, such as experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis(Larosa et al., 2019).

Non-steroidal Anti-inflammatory drugs (NSAIDs)

1.6 Introduction of non-steroidal Anti-inflammatory drugs

Non-selective non-steroidal anti-inflammatory drugs (NSAIDs) and selective cyclo-oxygenase inhibition NSAIDs (COXIBs) are commonly used to treat fever, pain, and rheumatoid arthritis (RA) and osteoarthritis. Cyclo-oxygenase (COX) has two isoforms, each of which is generated by a separate gene.COX-1 is a housekeeping gene that regulates many cell functions, including the complex set of mechanisms that protect the gastrointestinal mucosa from ulceration. It is found on chromosome 9 and operates as a housekeeping gene. In response to a range of inflammatory cytokines and damage, the COX-2 gene on chromosome 1 is immediate early and rapidly deregulate (Bacchi .S et al., 2012).

COX is divided into two isoforms, COX-1 and COX-2, each with its own tissue distribution and regulation.COX-1 is expressed in the background and is engaged in the creation of PG, which serves homeostatic functions, whereas COX-2 expression is increased during inflammation and other pathologic conditions, which serves pathologic functions. (Crofford et al., 2000).

The effects of NSAIDs on the various COX isoforms are measured in clinical trials.COX-2 inhibition by NSAIDs prevents prostaglandin formation at sites of inflammation or other types of tissue damage, whereas COX-1 suppression in other tissues – most notably platelets and

the gastroduodenal mucosa – can result in NSAID-related side effects like bleeding and gastrointestinal ulcer. (Lanas, 2009).

Additional factor added to this issue is the recognition that aspirin and NSAIDs, including Coxibs, may reduce the risk of colonic adenoma and colorectal cancer occurrence or recurrence; as a consequence the risk / benefit for gastrointestinal (GI) and CV events for those on low-dose aspirin and NSAIDs in a theoretically healthy population now confronts us (Lanza et al., 2009).

1.7 Classification of Non-steroidal Anti-inflammatory Drugs based on their Cyclooxygenase inhibition activity.

Group 1: COX-1 and COX-2 inhibitors with poor selectivity (5-fold COX-2 selectivity)

Ibuprofen, diclofenac, aspirin, piroxicam, and naproxen are examples of pain relievers.

Group 2: are capable of inhibiting both COX-1 and COX-2, with a preference for COX-2 inhibition (5 to 50 fold COX-2 selectivity) etodolac, celecoxib, meloxicam, nimesulide

Group 3: NSAIDs (>50-fold COX2 selectivity) that highly inhibit COX-2 but only faintly inhibit COX-1

Rofecoxib

Group 4: Sodium salicylate, nabumetone, and other NSAIDs in appear to be only mild inhibitors of both COX-1 and COX-2. (Bacchi. S et al., 2012).

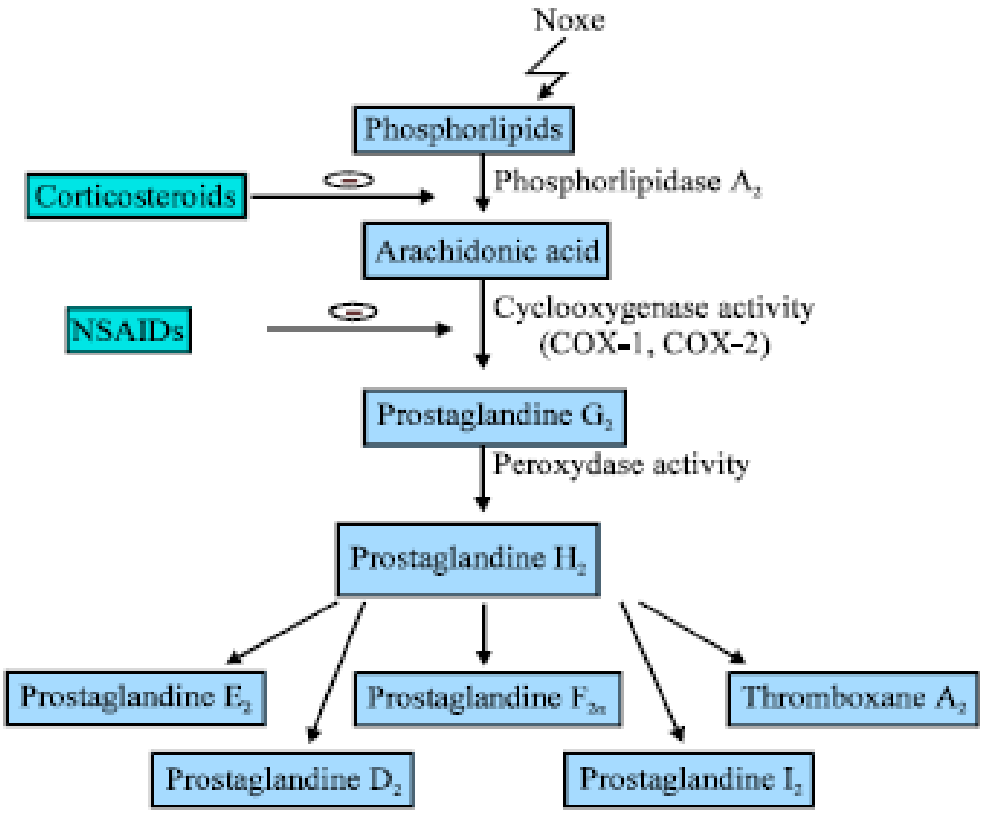
1.8 Mechanism of Action

The COX pathway produces prostaglandins (PGs), which are the end products of fatty acid metabolism. They have 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).

The COX pathway and the lipoxygenase (LOX) enzyme catalysis generate free arachidonic acid (AA) in response to a variety of stimuli, which is then transformed to lipid mediators known as eicosanoids. The first committed step in the synthesis of PGs and thromboxanes in the COX pathway is catalyzed by the two COX isoforms (COX-1 and COX2). COX reaction converts AA to prostaglandin G2 (PGG2), and the peroxidase reaction reduces PGG2 to prostaglandin H2 (PGH2), which is then converted into five biologically active primary PGs by cell specific isomerases and synthases: prostaglandin D2 (PGD2), prostaglandin E2 (PGE2), prostaglandin F2 (PGF2), and prostacyclin (PGI2) (Mattam et al., 2021).

After a clot forms, COX-1 inhibition is monitored as a function of platelet thromboxane synthesis. The inhibition of COX-2 is predicated on the inhibition of PGE2 production in a heparinized blood sample after LPS stimulation. A COX-2-selective NSAID has no effect on platelet COX-1 at concentrations equivalent to or greater than those that maximally inhibit COX-2 (Croff, 2013).



Figure(1.1) mechanism of non-steroidal anti-inflammatory drugs

1.9 Non-Steroidal Anti-inflammatory Drugs Effect

1.9.1 NSAID effect on inflammation

Prostaglandin (PG) are hormone-like compounds generated from arachidonic acid that play a role in a variety of physiological and pathological processes, including inflammation modulation. The production of prostaglandins is a major contributor to postoperative inflammation. The NSAID mode of action inhibits the cyclo-oxygenase system (COX-1 and COX-2), decreasing prostaglandin (PG) production and thereby reducing inflammation-promoting endogenous prostaglandin output. In the treatment of postoperative ocular inflammation, topically administered ophthalmic NSAIDs are commonly employed. To reduce inflammation after eye surgery, NSAIDs act in conjunction with corticosteroid therapy (Heier et al., 2000).

Posterisynechia, pseudophakic cellular precipitates, secondary glaucoma, and chronic uveitis have all been drastically decreased as a result of improved surgical techniques like as phacoemulsification and small incision surgery with foldable biocompatible intraocular lens materials. With the introduction of intraocular lenses that are specifically created and approved for refractive implantation, this is becoming a more severe issue. Surprisingly, research has showed that postoperative NSAID administration enhances treatment outcomes, despite advances in contemporary cataract surgical approaches (K. D. Solomon et al., 2001).

1.9.2 Inhibition of Cell Adhesion by Non-Steroidal Anti-inflammatory Drugs

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. The transmigration of leukocytes through the vascular endothelium and their accumulation in inflamed tissues is an early event required for an efficient inflammatory response. The adhesion cascade is a series of highly coordinated adhesion events between circulating leukocytes and endothelial cells that allows for cellular extravasation. The adhesion cascade is separated into four phases, each of which is required for leukocyte extravasation, therefore if one step is stopped, leukocyte emigration will be disrupted. Two types of NSAIDs have been demonstrated to exhibit antiadhesive effects in neutrophils, one interacting with the other. (Federico, 2015).

1.10 Therapeutic uses

1.10.1 Therapeutic effects of Non-Steroid Anti-inflammatory drugs in rheumatic diseases

NSAIDs are commonly used as first-line medications to relieve the symptoms of a variety of inflammatory diseases. NSAIDs have been compared to placebo, aspirin, and each other in double-blind, randomized clinical trials in inflammatory arthritis. The most common approach for clinical studies of NSAID efficacy in RA and OA is to cease the present NSAID and require the patient to show an increase in symptoms or flare to enter the study. Although NSAIDs can help with pain and stiffness, they don't usually diminish acute-phase reactants or change radiographic progression. NSAIDs have also been shown to have anti-inflammatory properties in OA, rheumatoid fever, juvenile rheumatoid arthritis, ankylosing spondylitis, gout, and systemic lupus erythematosus (Croff, 2013).

When administered in levels far lower than those required to reduce inflammation, all NSAIDs relieve pain. The suppression of PG synthesis in peripheral tissues and the central nervous system is what gives NSAIDs their analgesic effect. PGs do not cause pain in the periphery, but they do sensitize peripheral nociceptors to the actions of mediators like bradykinin and histamine (Ito et al., 2001).

The activation threshold of tetrodotoxin-resistant sodium channels on sensory neurons is lowered by PGs generated during inflammation or other damage. PGs have a significant part in neuronal sensitization in the central nervous system, where NSAIDs and acetaminophen have analgesic effects.COX-2 is expressed in the dorsal horn of the spinal cord on a constant basis, and its expression rises during inflammation (Yaksh et al., 2001).

NSAIDs act by inhibiting the activity of the cyclooxygenase (COX) enzymes and thus the conversion of arachidonic acid to several PG derivatives. These important immunomodulatory molecules (including PGD2, PGI2, PGF2a, thromboxane A2, and PGE2) can regulate many processes like pain, vasodilation, cardioprotection, apoptosis, and bone remodeling and are considered important in both normal homeostasis and many disease pathologies (Page et al., 2010).

1.10.2 Non-steroidal Anti-Inflammatory Drugs and Their Role in Cancer

1.10.2.1 Targeting Inflammation in Cancer.

Some of the targets examined in the battle against cancer-related inflammation include COX, NF-kB, cytokines/chemokines and their receptors, fibroblast growth factor (FGF) and its receptor, as well as a vascular endothelial growth factor. COX has been identified to be overexpressed in a variety of malignancies in recent decades, including pancreatic, prostate, cervical, breast, lung, and colon cancer. Angiogenesis, a crucial stage in invasion and metastasis, was discovered to be induced by COX overexpression. Overexpression of COX was previously discovered to be precancerous by increasing cancer cell resistance to apoptosis. As well-known COX inhibitors, NSAIDs are a prominent anticancer anti-inflammatory choice in cancer therapy and prevention. Because some of the products of COX activity (e.g., prostaglandin E2) are involved in carcinogenesis, inhibiting COX could be an effective technique. Prostaglandin E2 (PGE2) levels have been found to be higher in cancer cells, and it has been proven to promote cancer cell proliferation and invasion. The COX-2/PGE2 signaling pathway has been linked to the development of colorectal cancer. (Wong, 2019)

1.10.2.2 NSAIDS induce apoptosis

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). A critical event in apoptosis is the release of cytochrome c from mitochondria. The apoptosome complex is formed when cytochrome c released into the cytoplasm binds with the apoptotic protease activating factor-1, which leads to the successive activation of caspase-9 and caspase-3. Upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins is also a possible target for NSAIDs-mediated apoptosis. NSAIDs have been shown to down regulate Bcl-2- expression which Potential mechanisms of NSAIDs-induced apoptosis.(Jana, 2008)

1.10.2.3 Sulindac metabolites induce apoptosis in vitro and in vivo.

Sulindac is a pro-drug that is rapidly metabolized in vivo to 2 major metabolites. About 50% of the sulfoxide is initially converted by a reversible oxidation/reduction reaction to sulindac sulfide (SS), which is a potent anti-inflammatory drug (an NSAID) that inhibits prostaglandin synthesis by inhibiting both cyclooxygenase (COX) 1 and 2. The other 50% of the sulfoxide is irreversibly reduced to a sulfone. Because of the reversibility of the sulfide reaction, sulfone is ultimately the major sulindac metabolite. We and others have shown that both major sulindac metabolites inhibit the growth of a variety of cancer cell lines and that they induce apoptosis as well as cell cycle arrest. We have found that the major mechanism of the growth inhibition induced by sulindac metabolites is due to the induction of apoptosis and that cell-cycle arrest is not required for either the growth inhibition of apoptosis induced by the drugs (Ahnen, 1998).

1.11 Side Effect of Non-Steroidal Anti-inflammatory Drugs

1.11.1 Upper gastrointestinal effects of Non-Steroidal Anti-inflammatory Drugs

Upper gastrointestinal adverse events of NSAIDs can be categorized into different types:

1. Symptom like dyspepsia, nausea, vomiting, abdominal pain, and heartburn. They are the most usual adverse GI effects linked to NSAID use and can be present in up 40% of NSAID users.
2. NSAIDS related gastroduodenal injury with unclear clinical significance. This injury includes a combination of subepithelial hemorrhages, erosions, and ulcerations. This damage happens in 30–50% of patients taking NSAIDs but most lesions are asymptomatic, trifling, and disappear or reduce in number with continued use thanks probably due to the mucosal adaptation process.
3. Symptomatic Ulcers and Gastrointestinal Complications.
4. Mortality. The worst outcome of GI complications is death, but mortality data associated with NSAID treatment are scant up to one-third of all NSAID/aspirin deaths can be attributed to low dose aspirin use (Sostres et al., 2010).

1.11.1.1 Mechanism of Non-Steroidal Anti-inflammatory Drugs -Induced GI Injury

The suppression of the enzyme COX-1 and the gastro-protective PG, membrane permeabilization, and the generation of extra pro-inflammatory mediators are the three primary mechanisms of NSAID-induced GI problems.

1. COX-1 Inhibition and Gastro-protective

PGCOX has two isoforms, COX-1 and COX-2, each with its own set of functions. (Zidar et al., 2009).

COX-1 is a naturally occurring enzyme that provides physiological protection to the stomach mucosa. It makes bicarbonate and creates prostaglandins, which protect the stomach lining from released acid, keep blood flowing through the gastric mucosa, and protect the stomach lining from secreted acid (Sinha et al., 2013).

COX-2, on the other hand, is activated by cell injury, proinflammatory cytokines, and tumor-derived substances. The inhibition of COX-1 by NSAIDs is the principal cause of NSAID-induced gastropathy.

2. Membrane Permeabilization .

Gastric mucosal cells are also damaged by NSAIDs, resulting in ulcers and inflammation. In several studies, direct cytotoxicity was discovered to be independent of COX inhibition. Acidic NSAIDs like aspirin have been shown to cause topical damage, resulting in an accumulation of ionized NSAID, a condition known as "ion entrapment." NSAIDs are hypothesized to cause membrane permeabilization, which leads to the collapse of the epithelial barrier. NSAIDs were also found to promote necrosis and apoptosis in stomach muscle cells. (Sinha et al., 2013).

3. Production of Additional Pro-inflammatory Mediators.

The simultaneous stimulation of the lipoxygenase pathway and enhanced synthesis of leukotrienes occurs when NSAIDs inhibit prostaglandin production. Gastric mucosal damage is caused by

leukotrienes, which promote inflammation and tissue ischemia. (Santucci et al., 1994).

There is also an increase in the synthesis of proinflammatory mediators like tumor necrosis factors. This causes obstruction of stomach microvessels, which results in decreased gastric blood flow and the production of oxygen-derived free radicals. Lipid peroxidation and tissue injury occur when free oxygen radicals react with polyunsaturated fatty acids in the mucosa. (Wallace, 1997).

1.11.1.2 Mucosal Protection

In patients taking NSAIDs, two strategies are widely used to avoid the development of

1. peptic ulceration and mucosal damage: Co-treatment with a PPI, such as a high-dose (2 RA) histamine-2-receptor antagonist (H 2 RA) or misoprostol, a synthetic prostaglandin E1 analog.
2. Substitution of a COX-2 inhibitor for a typical NSAID:

Although co-treatment with a standard-dose H 2 RA has been demonstrated to prevent duodenal ulcers, it has not been proved to protect stomach ulcers caused by NSAIDs. Enteric coating or buffering of NSAIDs and co-therapy with sucralfate are not effective in preventing NSAID-related gastric or duodenal ulceration (Lanza et al., 2009).

1.12 Review about (Aspirin , Sulindac , Celecoxib)

1.12.1 Aspirin

Also known as acetylsalicylic acid, is a classic drug in the history of medicine and is widely used to treat fever and inflammation. It is usually used to treat or prevent heart attacks and stroke. The dose of

aspirin is from 75 to 300 mg to treat or prevent cardiovascular diseases. (Z. Tian et al., 2017)

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 prevention of thromboxane-A2 (TXA2) biosynthesis and thus inhibits platelet aggregation (Xiao et al., 2019).

Aspirin has been used as an analgesic, antipyretic and anti-inflammatory drug for many years. In general, orally administrated aspirin is rapidly and completely absorbed from the GI tract. The maximal plasma levels (Cmax) of aspirin are usually reached within less than 30 min. During intestinal uptake and liver passage ASA is enzymatically converted in first-pass metabolism to its main active metabolite salicylic acid (SA). Cmax of SA is reached in a dose dependent manner within 2–3 h. (Voelker & Hammer, 2012).

The t1/2 of ASA is about 15–20 min. ASA is rapidly decomposed into salicylic acid (SA) in the blood and gastrointestinal tract after giving medicine (Z. Tian et al., 2017).

Aspirin has an irreversible antiplatelet effect, while other NSAIDs, including ibuprofen and naproxen, have a reversible antiplatelet effect COX-1-inhibition is irreversible because aspirin permanently acetylates a strategically located serine residue on the platelet COX-1 enzyme. (Gurbel et al., 2018).

1.12.2 Celecoxib

Celecoxib was the first COX-2 inhibitor to be approved for use in clinical trials. Celecoxib is now licensed for the treatment of osteoarthritis

(OA) and rheumatoid arthritis (RA). Celecoxib is also approved in the United States for the relief of the signs and symptoms of juvenile rheumatoid arthritis and ankylosing spondylitis (AS), as well as the management of acute pain in adults and the treatment of primary dysmenorrhea; it is also approved in the United States and the European Union for the reduction of adenomatous colorectal polyps in familial adenomatous polyposis (Brauer, 2007).

In vitro and in vivo, celecoxib inhibits tumor initiation and tumor cell proliferation. Patients with the genetic family adenomatous polyposis (FAP) condition have a reduced incidence of colonic polyps and a lower risk of colon cancer, according to epidemiological research.

Celecoxib has also been shown in preclinical studies to increase the sensitivity of tumor cells to chemotherapy, radiation, and chemoradiotherapy. Celecoxib appears to be a promising anticancer drug based on these data. Celecoxib has been approved for oral use in the prevention of colon cancer in individuals with FAP, and clinical trials are being conducted to assess its therapeutic potential in the treatment of advanced human cancers (Jendrossek, 2013).

Over the therapeutic dosage range, celecoxib exhibits approximate dose-proportionality (up to 200mg twice daily). Peak plasma concentrations are reached 3 hours after an oral dose, while steady-state plasma concentrations take 5 days. The hepatic cytochrome P450 2C9 isoenzyme converts celecoxib into inactive metabolites, which are eliminated in the urine and feces. In patients with moderate hepatic impairment, the daily dosage should be lowered by half. Fluconazole and lithium have shown to have substantial pharmacological interactions with celecoxib. (Brauer, 2007).

For celecoxib, the increased cardiovascular risk seems to be exposure dependent; both the dose and the dosing interval may be

important factors in cardiovascular risk. In the Adenoma Prevention with Celecoxib (APC) trial, celecoxib 400 mg twice a day exhibited a greater than three-fold risk for combined endpoints of cardiovascular death, myocardial infarction, stroke, or heart failure compared with placebo, and 200 mg twice a day with a greater than two-fold risk. Patients with higher baseline cardiovascular risk factors also tended to exhibit an increased risk(Manuscript, 2013).

1.12.3 Sulindac

Sulindac sulfoxide is an NSAID that inhibits COX and reduces PG synthesis, making it useful in the treatment of chronic inflammatory illnesses like arthritis. It's a prodrug that can be reversibly reduced to a COX-inhibiting sulfide derivative or irreversibly oxidized to a COXinactive sulfone derivative.

Sulindac's reversible biotransformation in equilibrium with sulfide gives the active medication a lengthy half-life and good stomach tolerance. Sulindac also has pulmonary effects, as it reduced induced pulmonary fibrosis in rats. Sulindac reduced TNF-induced luciferase activity in HeLa cells and the lung epithelial cell line BEAS-2B in a dose dependent manner. (Rocca et al., 2016).

Sulindac metabolites have proven to be effective pharmacological probes for determining if COX inhibition is required for NSAID chemopreventive effects. Sulindac, as a sulfoxide prodrug, requires conversion to a sulfide that inhibits COX-1 and COX-2, and so has anti-inflammatory properties. The oxidation of the sulfoxide also produces a sulfone metabolite, which does not inhibit COX and does not contribute to sulindac's anti-inflammatory effect. Sulindac sulfone, like sulindac sulfide (SS), was shown to suppress colon carcinoma cell proliferation

and induce apoptosis in vitro, indicating a COX-independent mechanism (Piazza et al., 2009a)**.**

Sulindac is most often administered orally, but it has also been administered topically, intravenously, and rectally. Conventional regular release tablets are commercially available. Sulindac is 100 times more water-soluble than the sulfide metabolite**.** Sulindac and its sulfide and metabolites are extensively bound to plasma albumin, 93. 1, 95.4 and 97.9%, respectively. Sulindac sulphoxide is metabolised in the body by reduction to sulindac sulphide.. There is a low degree of participation of the cytochrome P450 isozymes in the metabolism of sulindac; however, catalysis in the presence of a NADPH or NADH generating system, and further stimulation by the flavin adenine dinucleotide (FAD)-containing monooxygenase, is evident Sulindac (Davies & Watson, 1997).

Chapter Two

Materials and Methods

Material and Methods

2.1Materials

2.1.1Chemicals:

The chemicals used in this study are listed in **(Table 2.1)** with their suppliers.

Table 2. 1 chemical used in the study

Chemical	**Company**	**Country**
Alcohol spray (ethanol 70%)	AMEYA FZE	UAE
Dimethyl sulfoxide (DMSO)	Roth	Germany
Fetal bovine serum (FBS)	Gibco	UK
Gentamycin (80 mg vial)	The Arab pharm.	Jordan
MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder	Roth	Germany
Phosphate buffer saline tablet	Gibco	UK

Roswell Park Memorial Institute-1640 (RPMI-1640) powder medium	Gibco	UK
Sodium bicarbonate powder	Ludo	Belgium
Trypsin-Ethylenediaminetetra acetic acid (EDTA) powder	US biological	USA

2.1.2 Instruments and Tools:

The instruments and tools used in the study are listed in **(Table 2.2)** with their suppliers.

Table 2. 2 List of Instruments and Tools Used in the Study

instrument or tool	Company	Country
Autoclave	Jeiotech	Korea
Automatic micropipettes (different sizes)	Human	Germany
Cell culture flask (25ml)	SPL	Korea
Cell culture plate (96-wells)	SPL	Korea

Digital camera	Sony	Japan
Distiller	ROWA	Germany
Double distillation water stills	GFL	Germany
Electric oven	Memmert	Germany
ELISA Reader	Human	Germany
(Sterile freezing vial (1.5 ml	Biofil	Australia
Incubator	Memmert	Germany
Inverted microscope	T.C Meiji techno	Japan
Laminar airflow cabinet	Labtech	Korea
Liquid nitrogen container GT38	Air Liquide	France
Magnetic stirrer	Labinco	Netherland
Microcentrifuge	Memmert	Germany
Millipore filter (0.45, 0.22μm)	Biofil	Australia
Whatman filter paper	Merck	Germany
pH Meter	WTW	Germany
Refrigerator	Arcelik	Turkey

Sensitive Balance	Labtech	Korea
Vortex	Kottermann	Germany
Water bath	Memmert	Germany

Table 2. 3 List of Drugs Used in the Study

drugs	**Company**	**Country**
Aspirin	Central Drug House(CDH)	India
Celecoxib	Central Drug House(CDH)	India
Sulindac	Central Drug House(CDH)	India

Table (2.4) list of ELISA assay kite used in our study

ELISA kit Interleukin 6	Elabscience	USA
ELISA kit Interleukin 12	Elabscience	USA
ELISA kit Tumor necrosis factor-α	Elabscience	USA

2.2 Methods

2.2.1 Preparation of chemicals

A-Phosphate Buffer Saline (PBS):

According to the Gibco manufacturer manual, the PBS was prepared by dissolving only one tablet in 500 ml of deionized distilled

water (DDW) with continuous stirring by a magnetic stirrer at room temperature resulting in a PH value of 7.45 without need for adjustment. Autoclaving is required for complete sterilization and stored in a closed bottle until used to keep sterile.

B-Gentamycin Stock Solution:

A gentamycin vial of 40 mg/ ml solution was considered as a stock solution and stored at a temperature of 4 C˚ until use. The final concentration of gentamycin in the medium was 50 µg/ml (Freshney, 2010).

C-Trypsin-(EDTA) Solution:

As indicated by US Biological headings, a weight of 10.1 gm of trypsin-EDTA powder dissolving in 0.9 Liter of double distilled water (DDW) with continuous mixing at room temperature. 7.2 of PH value should be reached and complete the volume to 1 Liter by DDW, the solution was sterilized through using millipore filters of 0.45 and 0.22 µm respectively, after that, the solution was kept at (- 20C˚) of temperature.

D- MTT Solution:

MTT powder (0.5 gm) was dissolved in PBS (100 ml) to achieve 5 mg/ml concentration. A 0.2 µm millipore filter was utilized to sterilize the MTT solution and stored in a sterile and light-protected bottle. The solution was stored at 4°C of temperature for multiple uses or at -20°C of temperature for long storage (Meerloo et al., n.d.)

2.2.2 Preparation of Tissue Culture Medium:

2.2.2.1 Preparation of Serum-Free Medium:

A-Liquid Roswell Park Memorial Institute (RPMI-1640) medium:

Liquid RPMI-1640 medium was prepared according to the Gibco product manual from RPMI-1640 medium powder as the following:

10.43 gm of RPMI-1640 medium powder was dissolved in 0.9 L of DDW in a volumetric flask. Other constituents added include: 2 gm sodium bicarbonate powder as needed and 80 mg of gentamycin were added with continuous stirring. The solution was completed to 1 liter by DDW with adjusting the PH at 7.4. Using 0.4 and 0.2 μm millipore filters respectively to sterilize the solution under the airflow cabinet. For examination of any contamination, 5 ml of the prepared medium was transferred to a sterile flask and incubated at 37 ºC for 4 days with continuous following up if there is no contamination, the medium could be stored at 4ºC temperature until use.

2.2.2.2Preparation of Serum-Medium:

Medium with serum was prepared as described in the preparation of serum-free medium in (2.2.2.1) in addition to 10 percent of fetal bovine serum.

2.2.2.3Preparation of Freezing Medium:

The freezing medium was prepared from the following compositions: 6 ml serum-free medium, 3 ml FBS, and 1 ml DMSO. The solution was stored at (- 20) C˚ temperature between uses (Meleady & O'Connor, 2006).

2.2.3 preparation of drugs

2.2.3.1 Aspirin

We weight 5mg of pure aspirin then dissolve it in 5ml of media (RPMI-1640) to obtain a stock solution of (1000μg\ml) and from this stock, the serial dilutions ware made.

2.2.3.2 Celecoxib

We weight 5mg of pure celecoxib then dissolved it in 1ml of DMSO then complete to5 ml by media (RPMI-1640) to obtain a stock solution of (1000 μg\ml) and from this stock, the serial dilutions ware made.

2.2.3.3 Sulindac

We weight 5mg of pure sulindac then dissolved it in 1ml of DMSO then complete to5 ml by media (RPMI-1640) to obtain a stock solution of (1000μg\ml) and from this stock, the serial dilutions ware made.

2.2.4 Cytotoxicity assay:

2.2.4.1 MTT Assay:

A-Principle:

The MTT test is commonly used to quantify live cells at fairly high throughput (96-well plates) without the need for complex cell counting. As a result, the most well-known application is to assess the cytotoxicity of a variety of medicines at varying concentrations. The MTT assay works on the assumption that most viable cell mitochondrial activity is constant, therefore any change in the number of viable cells is proportional to the mitochondrial activity. The change of the pale yellow colored tetrazolium salt (MTT dye) into other dark purple formazan

crystals by NADH, which may be solubilized for homogeneous measurement, reflects the mitochondrial activity of the cells. As a result, any rise or reduction in the number of live cells can be recognized by counting them formazan concentration reflected by optical density (absorbance) using a plate reader at 570 nm. The darker the solution, the greater the number of viable and metabolically active cells (Meerloo *et al.*, 2011)

B-Procedure:

1. The media was withdrawn from the wells at the end of the extract exposure period, and the cells were then washed with PBS. To assess unspecific formazan transformation, a blank control was performed.
2. To obtain a final concentration of 0.5 mg/ml, 1.2 ml of MTT solution (5 mg/ml) was added to 10.8 ml medium. After that, each well received 200 µl of the achieve solution.
3. Under the invertedi microscope, the 96-well plate was incubated for 3 hours at 37°C until intracellular purple formazan crystals were visible.
4. The supernatant was withdrawn, and 100µl DMSO was added to each well to dissolve the formazan crystals that had formed.
5. The 96-well plate was incubated at room temperature for 30 minutes until the cells have lysed and purple crystals had broken.
6. Microplate reader at 570 nm was used to evaluate the absorbance.

The blank and control readings of absorbance must be subtracted from each sample. Absorbance readings from samples should then be divided by those of the control and multiplied by 100 to get percentage cell proliferation or viability. Greater absorbance values compared to the control demonstrate cell proliferation, while lower values propose cell

death or inhibition of proliferation. Percentage of cell viability or percentage of inhibition was calculated by the following formula:

% viability = (Abs $_{sample}$ – Abs $_{Blank}$) / (Abs $_{control}$ – Abs $_{Blank}$) × 100%

% Inhibition = 100 – % viability (Meerlo *et al.*, 2011)

2.2.4.2 Preparation of Colon cancer (sw480) Cell Lines for Cytotoxicity Assays (Meleady & O'Connor, 2006):

The laboratory of tissue culture in the college of medicine/university of Babylon provided colon cell lines in frozen vials.

A-Harvesting and sub-culturing of Colon cancer (sw480) cell lines:

Harvesting is a procedure that uses proteolytic enzymes such as trypsin to detach and disaggregate the monolayer of adherent cells from the bottom of the flask of culturing. It was performed at whatever point the cells should be collected to be harvested for cell counting and sub-culturing of the cell line. This methodology was done according to the following:

1. The inverted microscope was utilized to analyze that the cells are healthy and sub-confluent without contamination.
2. The spent medium had been emptied by a pipette and added sufficient amount of pre-warmed trypsin- (EDTA) solution to wash the monolayer to guarantee the removal of all medium from the cell culture flask. This step could be repeated if the cells were still adherent strongly.
3. The convenient volume of trypsin- (EDTA) solution was included in the washed cell monolayer utilizing (1-2 ml) per 25-cm2 flask. Flask was rotated to completely cover the monolayer with trypsin.

4. To detach the cells from the surface of the flask, the flask was returned to the incubator at 37°C. Usually, the cell's detaching period depends on a cell line which could take 2 to 10 minutes.
5. An inverted microscope could be used to evaluate the cells whether they are detached and floating or not. The flask might be tapped gently on its side to detach any remaining cells.
6. An equal volume of serum-containing medium was added to deactivate trypsin in the flask.
7. If there are enough cells in the flask, an aliquot of cells could be transferred to another flask labeled with cell line name with a pre-warmed serum-containing medium (5–7ml for a 25-cm2 flask). DMEM was used for prostate PC3 cell line and RPMI medium used for colon HCT cell line and normal MDCK cell line.
8. The flask was incubated at 37C° temperature.
9. This process has been repeated according to the characteristics of the growth for each cell line.

B-Freezing of Colon cancer (sw480) cell lines:

The cell lines source were kept frozen at (-196) ºC in a nitrogen tank according to the following protocol:

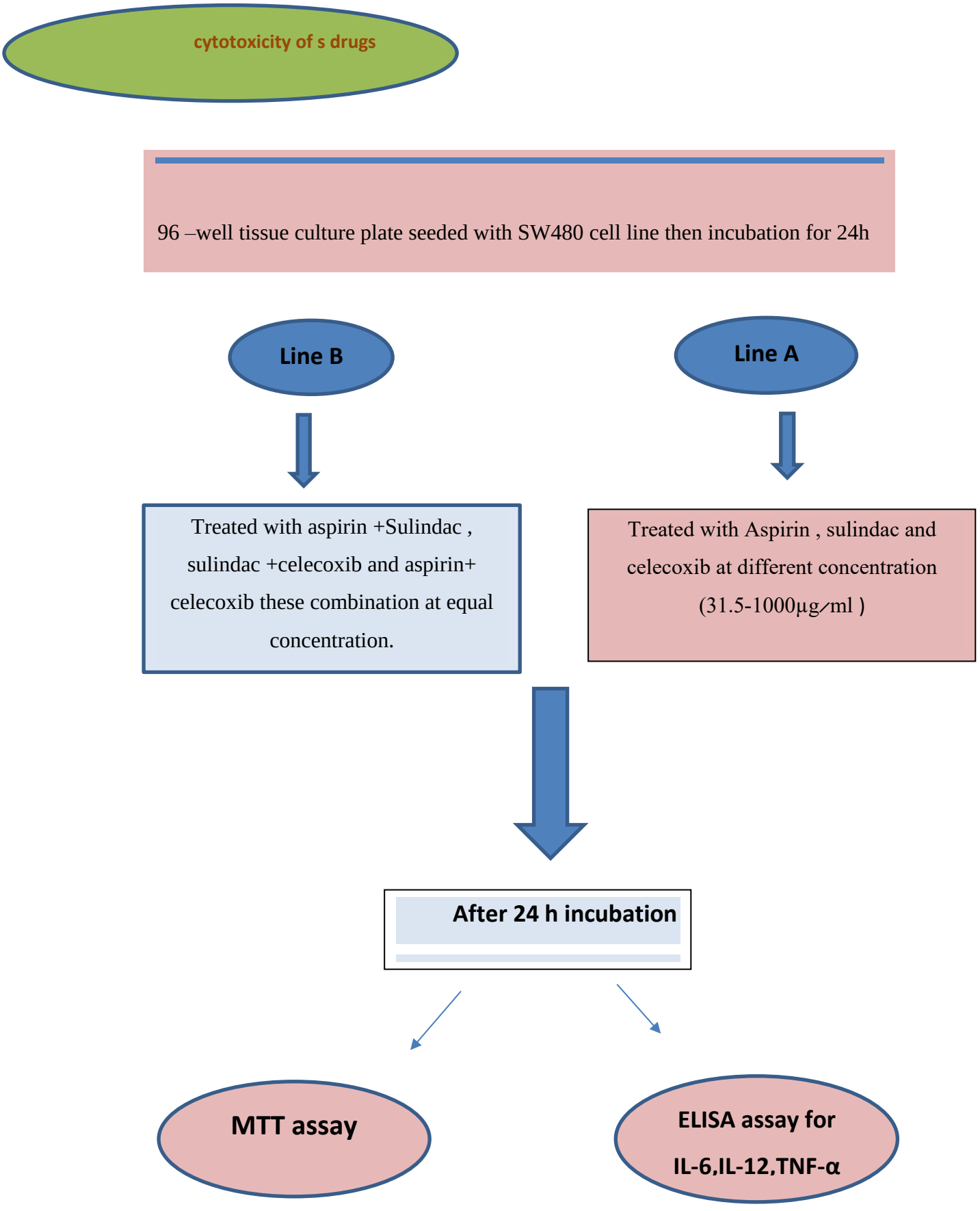
1. Cultures were checked to utilize an inverted microscope to evaluate the degree of cellular development and to guarantee that the cells are free of contaminations. Adherent cells are gathered (as mentioned above) for cryopreservation in the exponential phase of growth.
2. one ml of the freezing medium was included and then the content of the flask was moved into a 1.5 ml sterile freezing vial. All freezing vials were marked with the cell line name, date of freezing, and passage number.

3. These vials were put in the vapor phase of liquid nitrogen, which is equivalent to a temperature of -80°C for at least three hours (or overnight).
4. The vials were expelled from the vapor phase of the liquid nitrogen and moved to the liquid phase for storage (-196°C).

C-Thawing of Colon cancer (sw480) cell lines:

With caution, the frozen cell line vial was carefully removed from a liquid nitrogen container and placed into a beaker containing pre-warmed (37°C) sterile DDW. The vial was removed from the water before the ice floccule entirely disintegrated, and it was then cleaned with 70% ethanol. The vial's cell suspension substance was pipetted into a 15 ml sterile plastic centrifuge tube containing 10 ml of pre-warmed serum-free medium under a laminar stream cabinet. The supernatant was aspirated and decanted after centrifugation at 1000 rounds per minute for five minutes. The cells pellet was re-suspended in 5 mL warm (37C) serum media and transferred into a 25 mL cell culture flask, which was subsequently incubated at 37C with the serum medium refreshed. on the following day.

Plane of The Study



2.2.5 Experiments

Experiment No.1: The effect of Aspirin on colon cancer (SW480) cell lines at 24 and 48hours incubation time.

Colon cancer SW cell lines were seeded at a concentration of 5*105 cells in four columns of six duplicates of 96-well plates. Column No. 1 was used as a control group, with six replicates, and the three columns after that were exposed to Aspirin in serial dilutions of (2000,1000, 500, 250, 125, 62.5 µg/ml), with four duplicates for each concentration. The plate was incubated twice, once for 24 hours and the other for 48 hours, each time with a self-plastic lid. The wells were rinsed with 200 µl of sterile PBS when the exposure was completed. The MTT assay was used to analyze the effect of aspirin on the colon cancer line's proliferation.

Experiment No.2: The effect of Sulindac on colon cancer (SW480) cell line at 24 and 48hours incubation time.

Colon cancer (SW480) cell line was seeded in four columns of six repetitions of a 96-well plate at a concentration of 5*105 cells. Column No. 1 was used as a control group, with six replicates, and the three following columns were subjected to Sulindac in serial dilutions of (1000, 500, 250, 125, 62.5, 31.25 µg/ml), with four duplicates for each concentration. The plate was incubated twice, once for 24 hours and the other for 48 hours, each time with a self-plastic lid. The wells were rinsed with 200µ l of sterile PBS when the exposure was completed. The MTT assay was used to investigate the effect of Sulindac on the proliferation of a colon cancer cell line.

Experiment No.3: The effect of Celecoxib drug on colon cancer (SW480) cell line at 24 and 48hours incubation time.

Colon cancer(SW480) cell line was seeded in four columns of six repetitions of a 96-well plate at a concentration of 5*105 cells. Column No. 1 was used as a control group, with six replicates, and the three columns after that were exposed to Celecoxib in serial dilutions of (1000, 500, 250, 125, 62.5, 31.25 µg/ml), with four duplicates for each concentration. The plate was incubated twice, once for 24 hours and the other for 48 hours, each time with a self-plastic lid. The wells were rinsed with 200 µl of sterile PBS when the exposure was completed. The MTT assay was used to evaluate the effect of Celecoxib on the proliferation of a colon cancer cell line.

Experiment No.4: The effect of the combination of Aspirin and Sulindac drug on colon cancer (SW480) cell line at 24 and 48 hours incubation time.

Colon cancer(SW480) cell line was seeded in four columns of six repetitions of a 96-well plate at a concentration of 5*105 cells. Column No. 1 was used as a control group, with six replicates, and the three following columns were subjected to a mixture of aspirin and sulindac in serial dilutions of (1000, 500, 250, 125, 62.5, 31.25µg/ml), with four replicates for each concentration. The plate was incubated twice, once for 24 hours and the other for 48 hours, each time with a self-plastic lid. The wells were rinsed with 200 µl of sterile PBS when the exposure was completed. The MTT assay was used to analyze the effect of the medication combination on the colon cancer line's proliferation.

Experiment No.5: The effect of a combination of Aspirin and celecoxib drug on colon cancer (SW480) cell line at 24 and 48hours incubation time.

Colon cancer(SW480) cell line was seeded in four columns of six repetitions of a 96-well plate at a concentration of 5*105 cells. Column No. 1 was used as a control group, with six replicates, and the three following columns were subjected to a combination of aspirin and celecoxib in serial dilutions of (1000, 500, 250, 125, 62.5, 31.25µ g/ml), with four replicates for each concentration. The plate was incubated twice, once for 24 hours and the other for 48 hours, each time with a self-plastic lid. The wells were rinsed with 200 µl of sterile PBS when the exposure was completed. The MTT assay was used to analyze the effect of the medication combination on the colon cancer line's proliferation.

Experiment No.6: The effect of a combination of Sulindac and celecoxib drug on colon cancer (SW480) cell line at 24 and 48hours incubation time.

Colon cancer(SW480) cell line was seeded in four columns of six repetitions of a 96-well plate at a concentration of 5*105 cells. Column No. 1 was used as a control group, with six replicates, and the three following columns were subjected to a combination of Sulindac and Celecoxib in serial dilutions of (1000, 500, 250, 125, 62.5, 31.25µ g/ml), with four duplicates for each concentration. The plate was then covered with a self-plastic lid and incubated for 24 hours and 48 hours, respectively. The wells were rinsed with 200 l of sterile PBS when the exposure was completed. The MTT assay was used to analyze the effect of the medication combination on the colon cancer line's proliferation.

2.2.6 Elisa Kit

Assay procedure

1. Determine wells for diluted standard, blank, and sample. Added 100 µ L each dilution off standard, blank, and sample into the appropriate wells (it is recommended that all samples and standards be assayed in duplicate). Covered the plate with sealer provided in the kit. Incubated for 90 min at 37^0 C. The solution should be added to the bottom of micro ELISA plate well, to avoid touching the inside wall and causing foaming as much as possible.
2. Removed the liquid from each well and decanted it; did not wash Each well was immediately filled with 100 mL of Biotinylated Detection Ab working solution. A new sealer was applied to the plate. At 370 C, incubated for 1 hour.
3. Removed the solution from each well and replaced it with 350µL of wash buffer. After 1 minute of soak time, aspirate or decant the solution from each well and pat dry with clean absorbent paper. This wash phase was repeated three times. This and other wash stages can be done with a microplate washer. After the wash process, use the tested strips right away. Allowing wells to dry out is not a good idea.
4. Added 100µL of HRP conjugate working solution to each well. covered the plate with a new sealer. Incubated for 30 min at 37^0C.
5. Decanted the solution from each well, repeated the wash process 5 times as conducted in step 3.
6. Added 90µL of Substrate Reagent to each well. Covered the plate with a new sealer. Incubated for about 15 min at 37^0 C. Protected the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but no more than 30 min.

preheated the microplate Reader for about 15 min before OD measurement.

7. Fill each well with 50 µL of Stop Solution. It's important to add the stop solution in the same sequence as the substrate solution

8. Using a microplate reader set to 450 nm, determined the optical density (OD value) of each well at the same time.

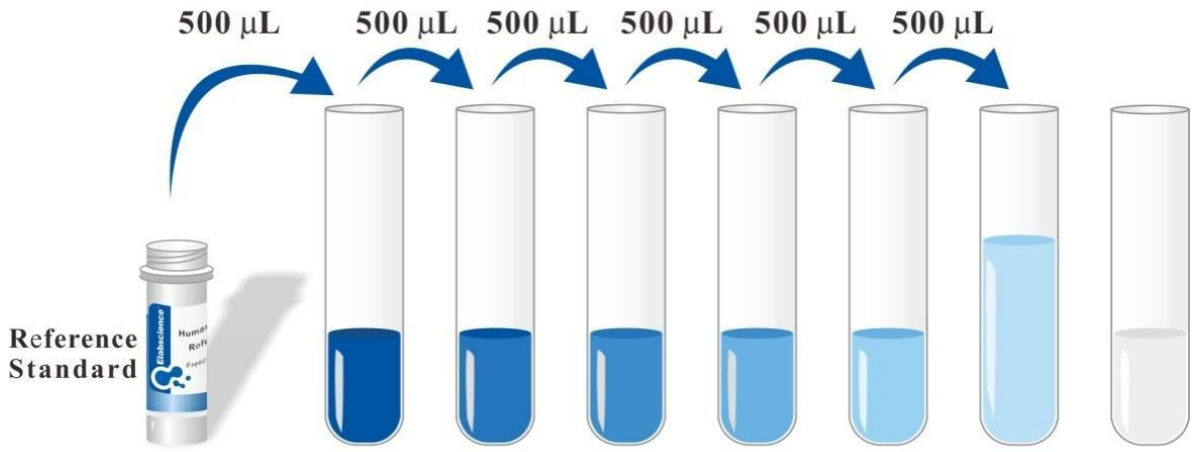
Test principle: This ELISA kit uses Sandwich-ELISA as the method. The micro-ELISA plate provided in the kit has been pre-coated with an antibody specific to the test of interest. Standards or samples are added to the appropriate micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for test of interest and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain test or protein of interest, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of test of interest. You can calculate the concentration of test of interest in the samples by comparing the OD of the samples to the standard curve. Reagent preparation Bring all reagents to room temperature (18-25℃) before use.

Wash Buffer - 30 mL of Concentrated Wash Buffer is diluted into 750 mL of Wash Buffer with deionized or distilled water. Then the unused solution is placed back at 4℃. If crystals have

formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard –standard is prepared within 15 minutes before use and centrifuged at 10,000×g for 1 minute, then reconstitute the Standard with 1.0 mL of Reference Standard & Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 10ng/ml. Then make serial dilutions as needed (making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows:10、5、2.5、1.25、0.625、0.313、0.156、0 ng/ml. If you want to make standard solution at the concentration of 5ng/mL, you should take0.5mL standard at 10ng/mL, add it to an EP tube with 0.5mL Reference Standard &Sample Diluent, and mix it. Procedures to prepare the remained concentrations are all the same. The undiluted standard serves as the highest standard (10ng/mL). The Reference Standard &Sample Diluent serves as the zero (0 ng/mL).

(Standards can also be diluted according to the actual amount such as 200μL/tube)



1	2	3	4	5	6	7	8	tube
10	5	2.5	1.25	0.625	0.313	0.156	0	ng/mL

serial dilution of reference reagent

Biotinylated Detection Ab – the required amount is calculated before experiment (100µL/well). Then the stock tube is centrifuged before use, and the concentrated Biotinylated Detection Ab is diluted to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate –the required amount is calculated before experiment (100µL/well). the Concentrated HRP Conjugate is diluted to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent: As it is sensitive to light and contaminants, so the vial should not be opened until there is a need. The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent should not be dumped back into the vial again.

Washing Procedure:

1. **Automated Washer**: 350µL wash buffer is added into each well, the interval between injection and suction should be set about 60s.

2. **Manual wash**: 350µL Wash Buffer is added into each well, then soaked for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

2.2.7 Statistical Analysis

All data were collected and analyzed by Microsoft Office Excel 2016 and Sigma plot version 12.5 software. ANOVA one way test was used to assess significant differences among the means of data. The p-value ($p<0.05$) were considered statistically significant.

Chapter Three

Results

3-Results

Table (3-1) percentage of cell viability in SW 480 colorectal cell line treated with different concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	Percentage of cell viability ±SD
Aspirin	Control	100±4.7664
	31	75.54931±10.93997*
	62	99.51222±8.178442
	125	107.0179±4.134194
	250	107.3255±2.969568
	500	97.45122±8.96998
	1000	91.20671±5.643978
Sulindac	Control	100±4.7664
	31	63.18334±13.07384*
	62	66.87467±8.704881*
	125	67.01822±4.760598*
	250	59.55352±12.3259*
	500	48.43851±16.94982*
	1000	37.52856±15.73998*
Celecoxib	Control	100±10.18769
	31	71.58704±11.72297*
	62	63.85584±6.822362*
	125	59.50092±6.323152*
	250	58.37549±5.80173*
	500	55.31726±2.401393*
	1000	61.72731±6.772738*

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

3.1. Effect of Aspirin on the viability of SW colon cancer cell line.

The result showed that there was a significant decrease in cells viability at the concentration of (31µg/ml) of aspirin. Results also showed a non-significant increase in cells proliferation at concentrations of (125 and 250) µg/ml after a period of 24hrs in comparison with the control group as indicated in Figure (3.1) and as shown in table (3-1).

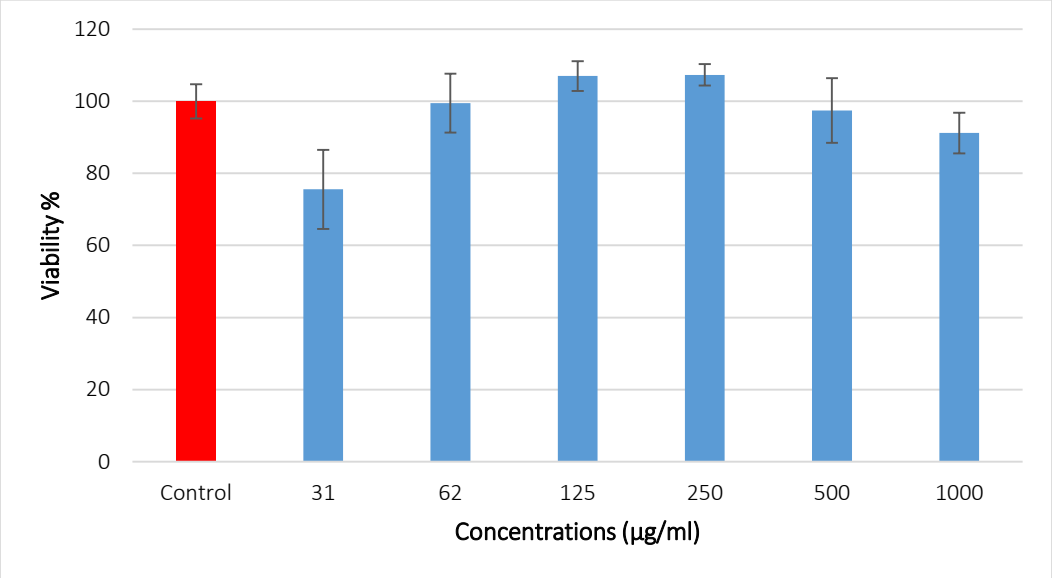


Figure (3.1): Effect of different concentrations of Aspirin on the viability of SW colon cancer cell line.

3.2 Effect of sulindac on the viability SW colon cancer cell line.

The results showed a highly significant decrease in cells viability for all concentrations used after 24hrs incubation in comparison with the control group as indicated in Figure (3.2) and as shown in table (3-1).

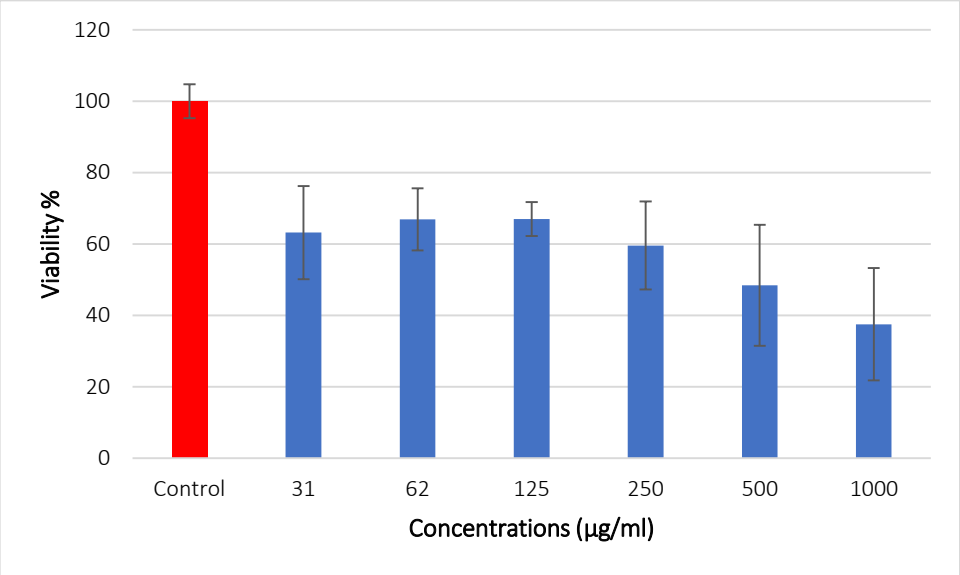


Figure (3.2): Effect of different concentrations of Sulindac on the viability of SW colon cancer cell line.

3.3 Effect of Celecoxib on the viability SW colon cancer cell line.

The result showed that there was a highly significant decrease in cells viability for all concentrations used after incubation for 24hrs in comparison with the control group, as shown in Figure (3.3) and as shown in table (3-1).

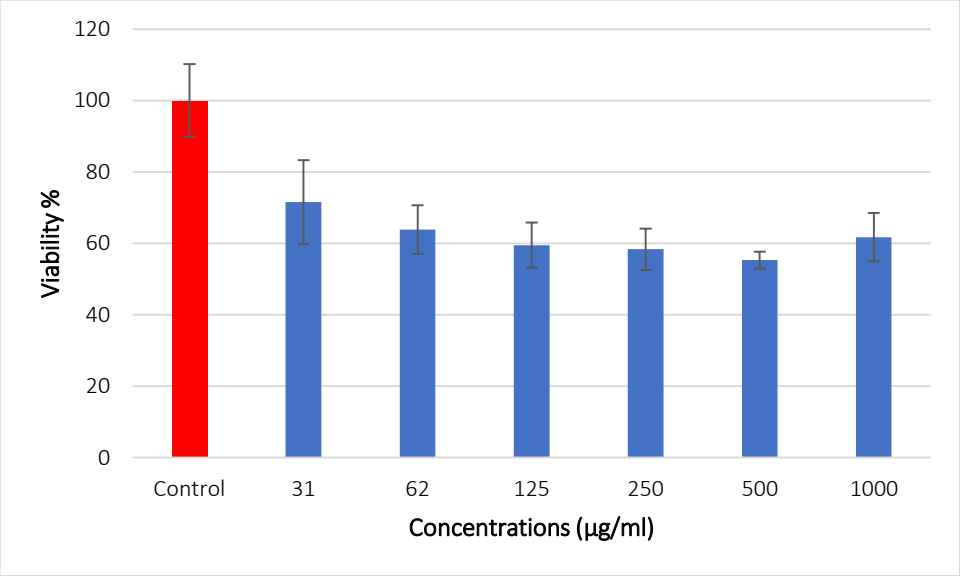


Figure (3.3): Effect of different concentrations of Celecoxib on the viability of SW colon cancer cell line.

Table (3-2) percentage of cell viability in SW 480 colorectal cell line treated with combination of equal concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	Percentage of cell viability ±SD
Aspirin and Sulindac	Control	100 ±11.84295
	31	124.0084±14.84738
	62	105.371±9.301694
	125	105.2192±16.22077
	250	79.3699±10.33297*
	500	72.99298±6.456849*
	1000	48.92769±0.817351*
Aspirin and celecoxib	Control	100±3.447216
	31	80.73162±3.334324*
	62	81.09974±2.074611*
	125	64.14356±5.384822*
	250	43.32221±11.96447*
	500	22.66191±1.532961*
	1000	29.03486±0.98546*
Sulindac and celecoxib	Control	100±2.442887
	31	76.77492±3.954724*
	62	77.514±2.13811*
	125	60.08959±5.352439*
	250	30.95185±10.88755*
	500	22.32923±4.399541*
	1000	27.83875±1.156549*

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

3.4 Effect of combination of Aspirin and Sulindac on the viability on SW colon cancer cell line.

The result showed a highly significant decrease in cell viability for the concentrations (1000,500,250 µg/ml), while other concentrations (125,62.5 and 31.25) showed a non significant increase in cell viability in comparison with the control group after incubation for 24 hrs, as shown in Figure (3.4) and as shown in table (3-2).

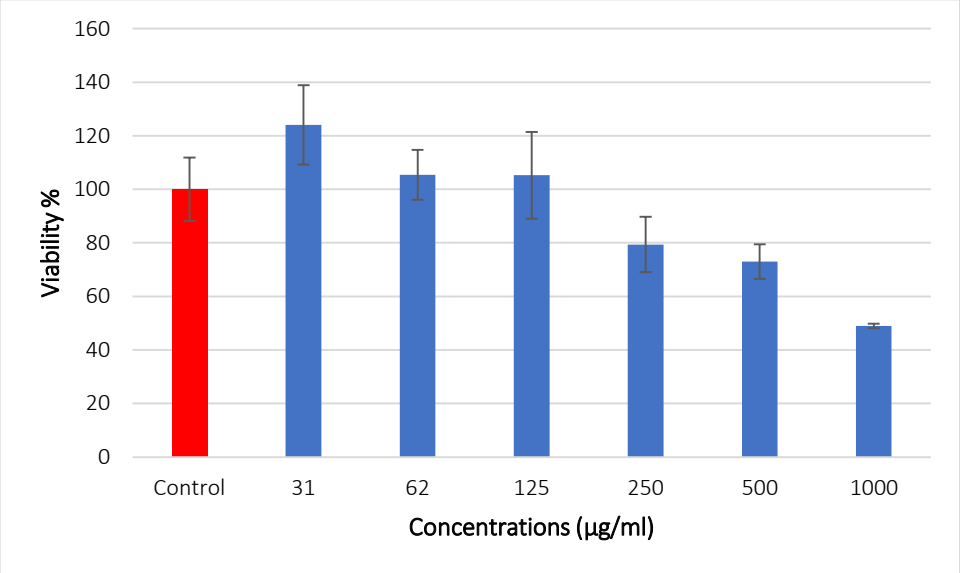


Figure (3.4): Effect of (Aspirin-Sulindac) combination at the equal concentration on the viability of SW colon cancer cell line.

3.5 Effect of Aspirin - Celecoxib combinations on the viability of SW colon cancer cell line

The result showed a highly significant decrease in cell viability for all concentrations used after incubation for 24hrs with the drug in comparison with the control group, as shown in Figure (3.5) and as shown in table (3-2).

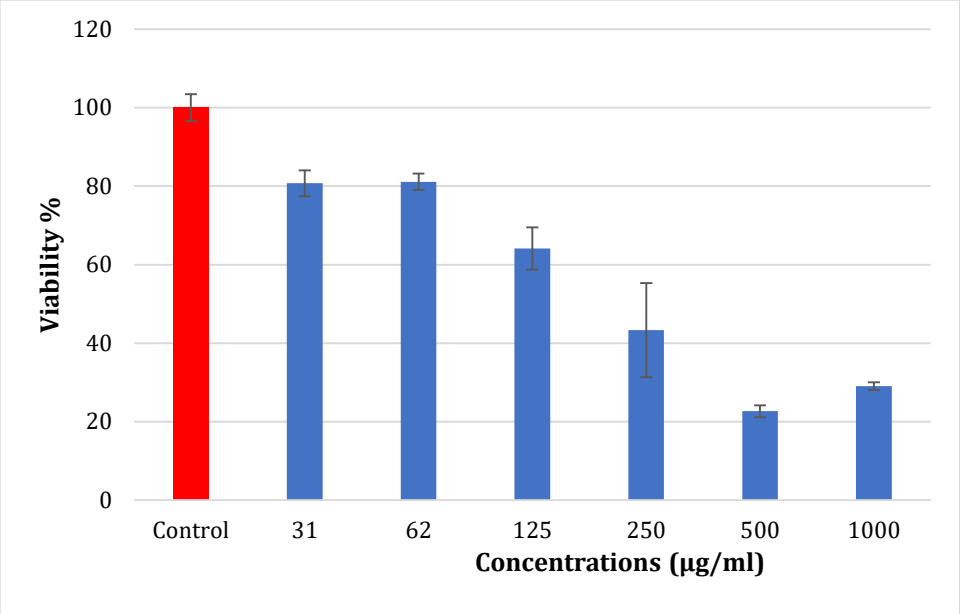


Figure (3.5): Effect of (Aspirin – Celecoxib) combinations at equal concentrations on the viability of SW colon cancer cell line

3.6 Effect of Sulindac - Celecoxib combinations on the viability of SW colon cancer cell line.

The result showed a highly significant decrease in cell viability for all concentrations after incubation for 24hrs in comparison with the control group, as shown in Figure (3.6) and as shown in table (3-2).

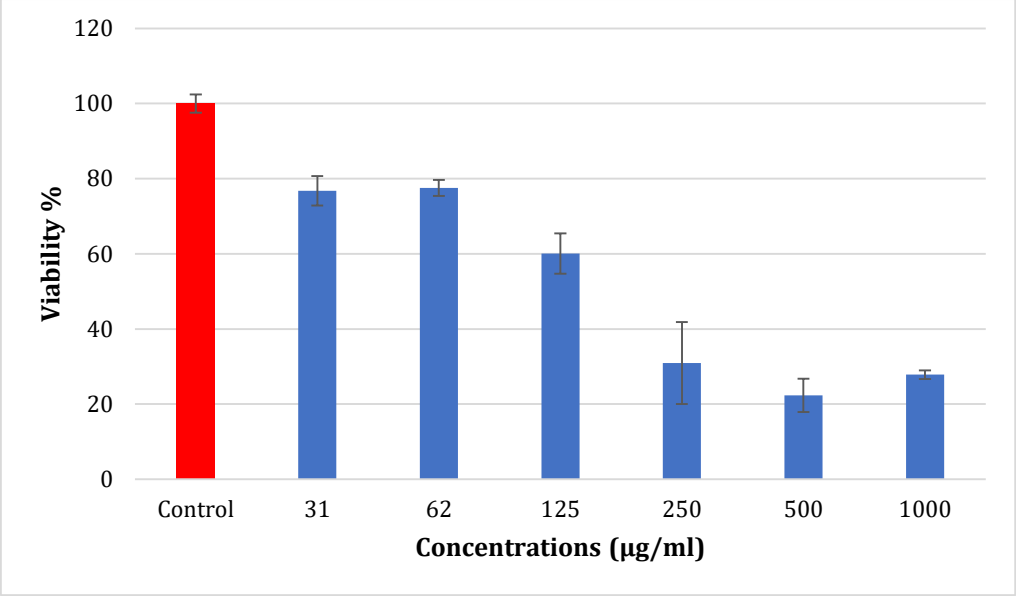


Figure (3.6): Effect of Sulindac - Celecoxib combinations at equal concentrations on the viability of SW colon cancer cell line.

Table (3-3) IL-12 level in SW 480 colorectal cell line treated with different concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	IL-12 concentrations (pg/ml) ±SD
Aspirin	control	8.216±0.98592
	62	8±0.96
	125	8.022±0.96264
	250	7.8±0.936
	500	8.194±0.98328
	1000	7.8±0.936
	2000	8.194±0.98328
Celecoxib	Control	10.113±1.21356
	31	9.747±1.16964
	62	9.2±1.104
	125	9.251±1.11012
	250	7.8±0.702
	500	8.173±0.73557
	1000	8.263±0.74367
Sulindac	Control	8.216±1.06808
	31	8.148±1.05924
	62	7.8±1.014
	125	9.876±1.28388
	250	8.8±1.144
	500	9.811±1.27543
	1000	14.059±1.82767*

Table (3-4) IL-6 level in SW 480 colorectal cell line treated with different concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	IL-6 concentrations (ng/ml) ±SD
Aspirin	control	8.899 ±0.44495
	62	8.28 ±0.414
	125	8.334 ±0.4167
	250	8.047 ±0.40235
	500	8.177 ±0.40885
	1000	8.494 ±0.4247
	2000	8.371 ±0.41855
Celecoxib	Control	10.251 ±0.51255
	31	8.912 ±1.06944
	62	8.989 ±1.07868
	125	8.802 ±1.05624
	250	8.76 ±0.5256
	500	8.13 ±0.4065*
	1000	8.337 ±0.58359*
Sulindac	Control	8.899 ±0.44495
	31	8.498 ±0.59486
	62	8.13 ±0.5691
	125	8.177 ±0.57239
	250	8.514 ±0.59598
	500	9.58 ±0.479
	1000	9.553 ±0.47765

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

Table (3-5) TNF-α- level in SW 480 colorectal cell line treated with different concentration of aspirin ,sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	TNF-α concentrations (ng/ml) ±SD
Aspirin	control	7.2 ±0.36
	62	7.1 ±0.71
	125	7.2 ±0.72
	250	7.3 ±0.73
	500	7.2± 0.72
	1000	8.04 ±0.4824
	2000	7.2 ±0.72
Celecoxib	Control	10.077±1.0077
	31	11.714±1.63996
	62	11.381±1.59334
	125	10.25±1.435
	250	9.744±1.36416
	500	9.318±1.30452
	1000	10.902±1.52628
Sulindac	Control	7.2±0.72
	31	7.2±0.72
	62	7.1±0.71
	125	7.1±0.71
	250	8±0.8
	500	14.136±1.4136*
	1000	9.757±0.9757*

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

3.7 Effect of Aspirin treatment on the cytokines production.

The result showed that there was no statistically significant effect of the Aspirin treatment on the production of IL-6, IL-12, and TNF- α in comparison to the control group as shown in figure (3.7) (3.8) and (3.9) respectively and as shown in table (3-3) (3-4) and (3-5) respectively.

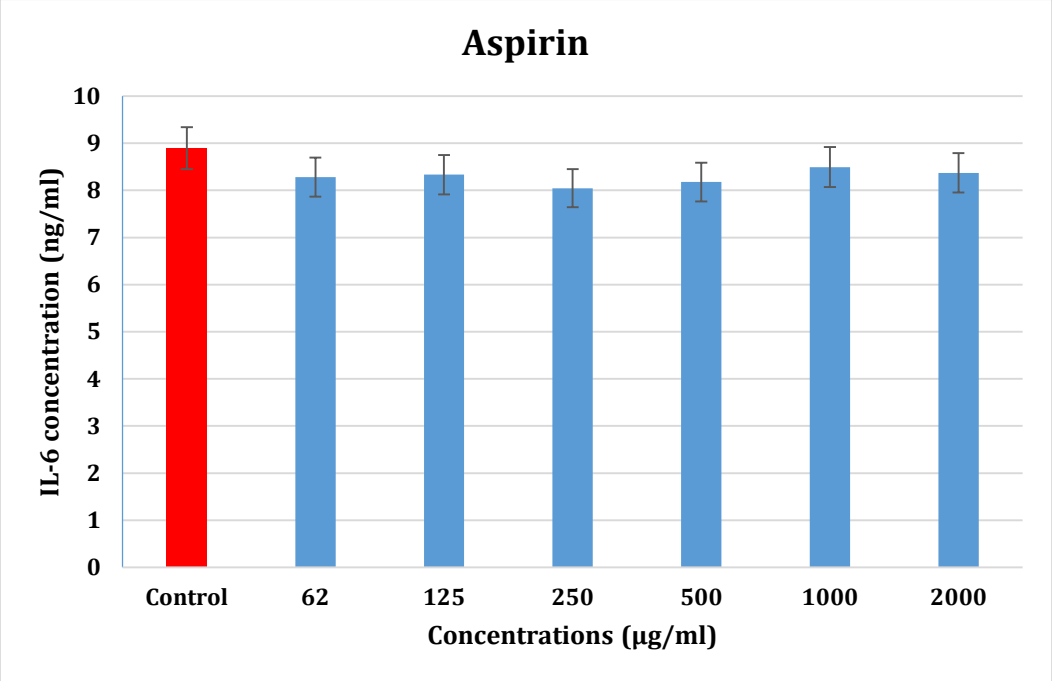


Figure (3.7): Effect of different concentrations of Aspirin drug on IL-6.

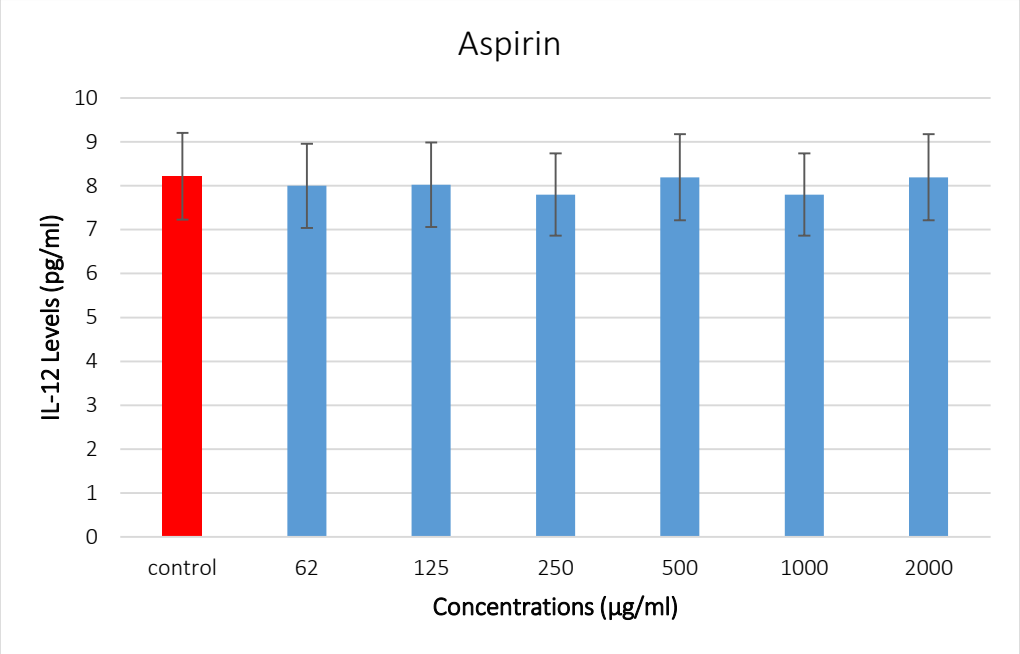


Figure (3.8) Effect of different concentration of Aspirin on IL-12.

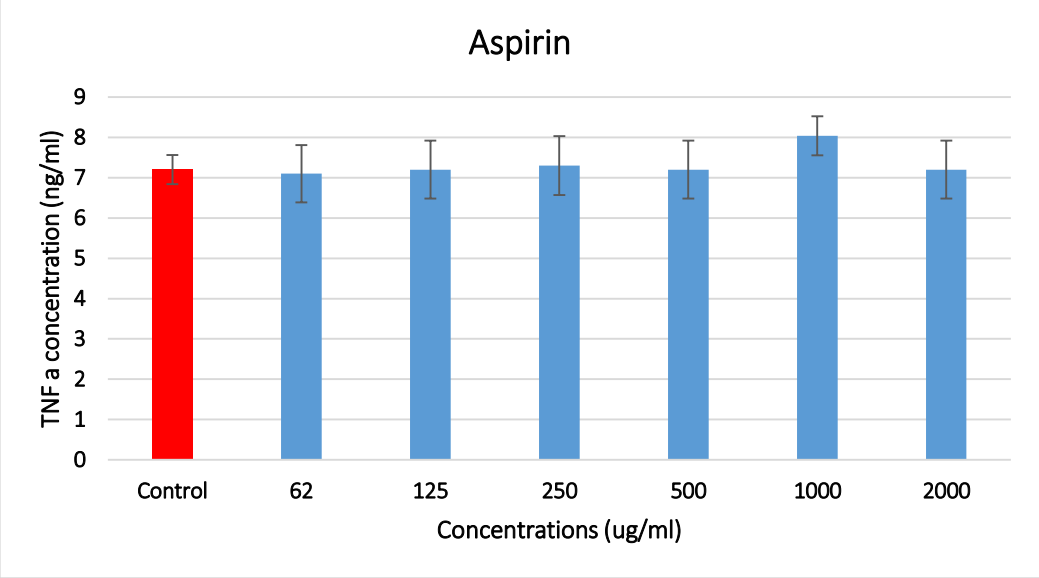


Figure (3.9): Effect of different concentrations of Aspirin on TNF-α.

3.8 Effect of Celecoxib drug on cytokine production.

The result showed that was a statistically significant decrease when using Celecoxib drug for concentration (500, 1000 µg/ml) on IL-6, while there was no statistically significant effect of the Celecoxib treatment on the production of IL-6 for concentration (31,62,125,250 µg/ml), IL-12 and TNF- α for all concentration in compared to the control group as shown in figure (3.10) , (3.11) and (3.12) respectively and as shown in table (3-3) (3-4) and (3-5) respectively.

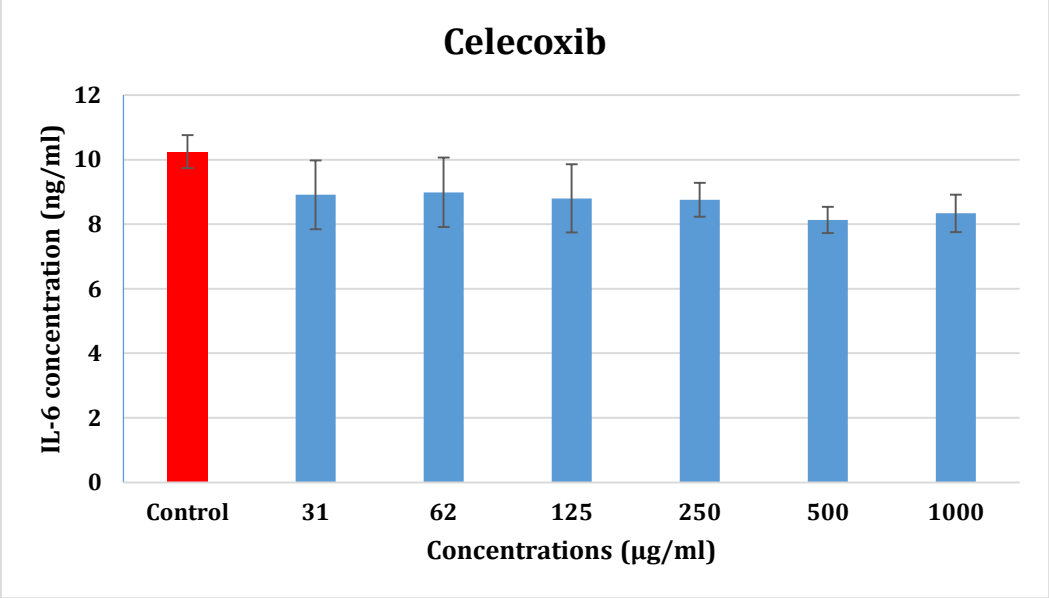


Figure (3.10): Effect of different concentrations of celecoxib drug on IL6.

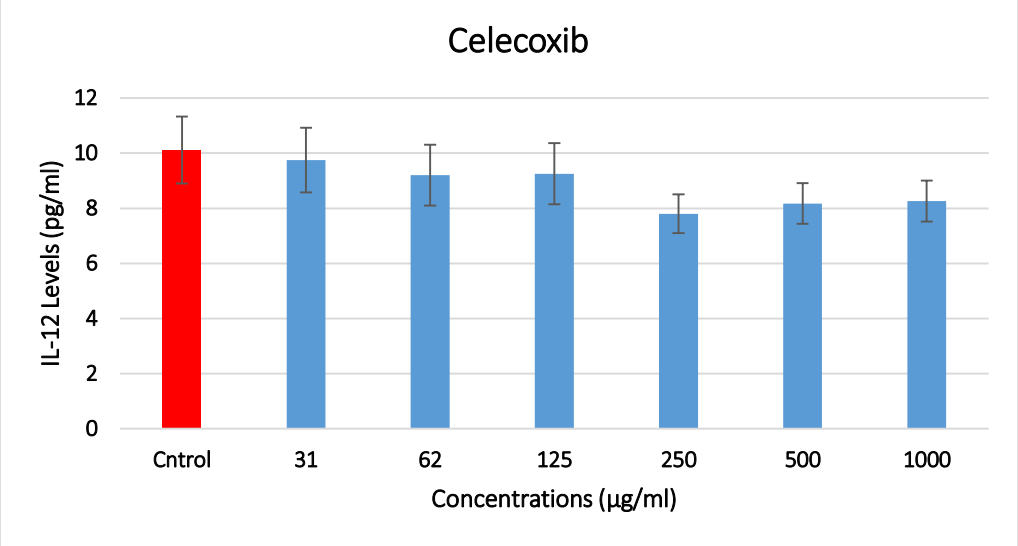


Figure (3.11): Effect of different concentrations of celecoxib drug on IL-12.

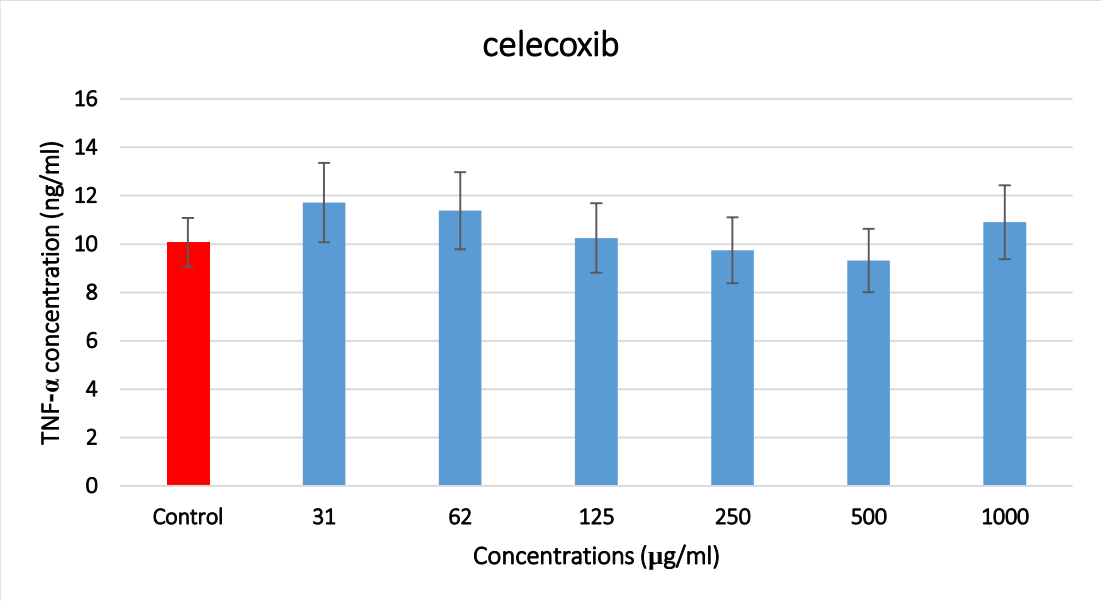


Figure (3.12): Effect of different concentrations of celecoxib drug on (TNF-α).

3.9 Effect of Sulindac drug on cytokine production.

The result showed that there was no statistically significant effect of the Sulindac treatment on the production of IL-6 and IL-12.

While there was a statistically significant increase for concentration 1000µg/ml when used sulindac drug on the production of IL-12 and in used Sulindac drug on the production of TNF-α for concentration (500, 1000µg/ml) in comparison with the control group as shown in figure (3.13), (3.14) and (3.15) respectively and as shown in table (3-3) (3-4) and (3-5) respectively.

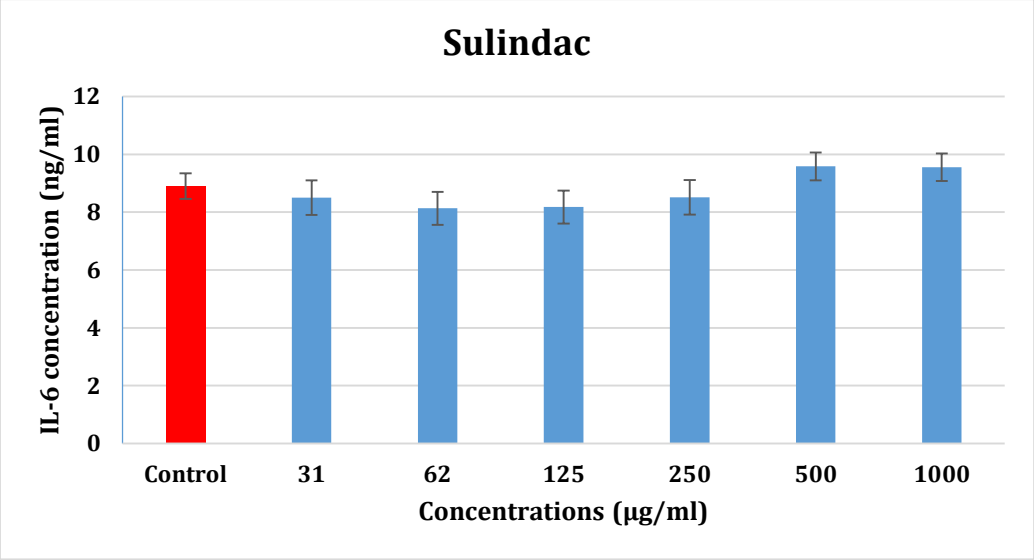


Figure (3.13) Effect of different concentration of Sulindac drug on IL-6.

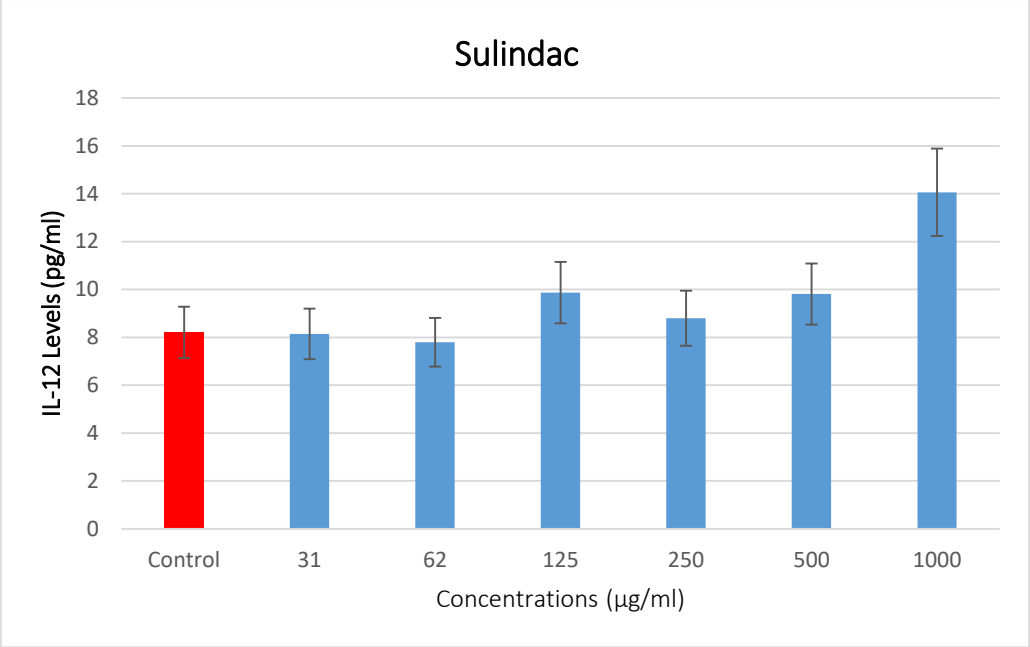


Figure (3.14): Effect of different concentration of Sulindac drug on IL-12.

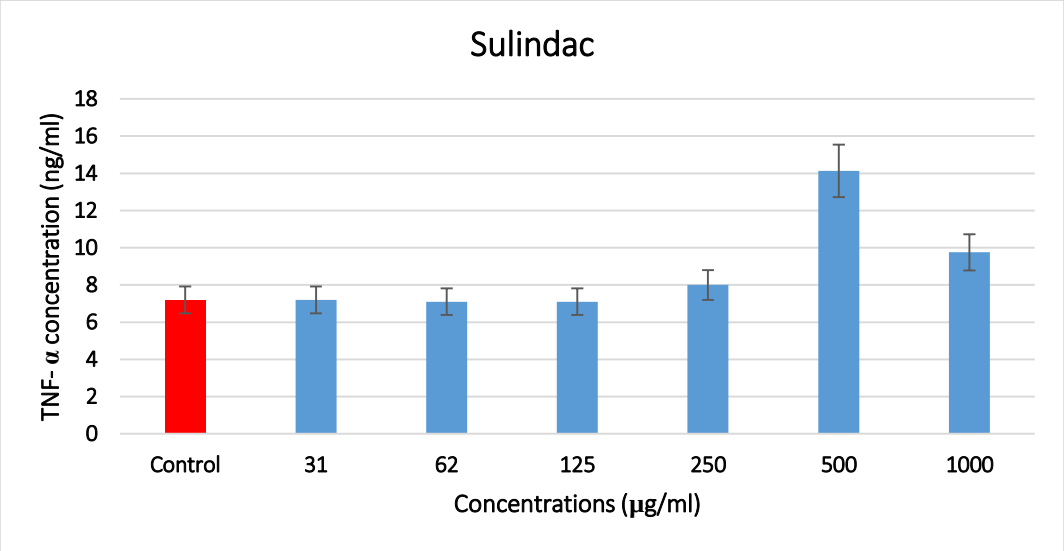


Figure (3.15): Effect of different concentrations of Sulindac drug on TNF-α.

Table (3-6) IL-12 level in SW 480 colorectal cell line treated with combination at equal concentration of aspirin ,sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	IL-12 concentrations (pg/ml) ±SD
Aspirin + Sulindac	control	8.496±1.2744
	31	9.639±1.44585
	62	9.229±1.38435
	125	8.647±1.29705
	250	8.2±1.23
	500	10.997±1.64955
	1000	11.105±1.66575
Aspirin + Celecoxib	Control	7.5±0.979
	31	8.388±1.00656
	62	8.755±1.0506
	125	8.582±1.02984
	250	8.129±0.97548
	500	8.065±0.9678
	1000	7.7±0.924
Sulindac + Celecoxib	Control	7.5±0.975
	31	6.1±0.793
	62	8.69±1.1297
	125	14.924±1.4924*
	250	27.602±3*
	500	8.129±1.05677
	1000	11.666±1.51658*

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

Table (3-7) IL-6 level in SW 480 colorectal cell line treated with combination at equal concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	IL-6 concentrations (ng/ml) ±SD
Aspirin + Sulindac	control	11.02±1.3224
	31	9.857 ±1.18284
	62	9.8456±1.181472
	125	9.9196±1.190352
	250	11.053±1.32636
	500	10.041±1.20492
	1000	8±0.96
Aspirin + Celecoxib	Control	8.688±1.3
	31	8.688±1.3032
	62	8.434±1.2651
	125	8.895±1.33425
	250	8.635±1.29525
	500	8.214±1.2321
	1000	8.845±1.32675
Sulindac + Celecoxib	Control	8.688±1.12944
	31	10.622±1.38086
	62	8.935±1.16155
	125	9.857±1.28141
	250	8.865±1.15245
	500	9.213±1.19769
	1000	9.617±1.25021

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

Table (3-8) TNF-α level in SW 480 colorectal cell line treated with combination at equal concentration of aspirin , sulindac , celecoxib as compared to control group

Treatment groups	Drug concentrations (µg/ml)	TNF concentrations (ng/ml) ±SD
Aspirin + Sulindac	control	8.679 ±0.69432
	31	10.542 ±0.84336*
	62	10.425 ±0.834*
	125	11.647 ±0.93176*
	250	11.797 ±0.94376*
	500	12.486 ±0.99888*
	1000	12.1 ±0.605*
Aspirin + Celecoxib	Control	8.466 ±0.4233
	31	9.797 ±0.68579*
	62	9.757 ±0.68299*
	125	9.525 ±0.66675
	250	9.491 ±0.66437
	500	8.5 ±0.595
	1000	8.546 ±0.59822
Sulindac + Celecoxib	Control	8.466 ±0.50796
	31	8.624 ±0.51744
	62	8.1 ±0.486
	125	8.546 ±0.51276
	250	7.91 ±0.791
	500	7.8 ±0.78
	1000	11.847 ±1.1847*

* Data are presented as mean ± SD

* Significant difference ($p<0.05$) compared with control group

3.10 Effect of Aspirin - Celecoxib combinations on cytokine production.

The result showed that there was no statistically significant effect of the Aspirin- Celecoxib combination treatment on the production of IL-6, IL-12. While there was a statistically significant increase for concentration (31,62µg/ml) and no statistically significant effect for concentration (125,250,500.1000µg/ml) of Aspirin - Celecoxib combinations on the production of TNF-α compared to the control group as shown in figure (3.16) (3.17) and (3.18) respectively and as shown in table (3-6) (3-7) and (3-8) respectively.

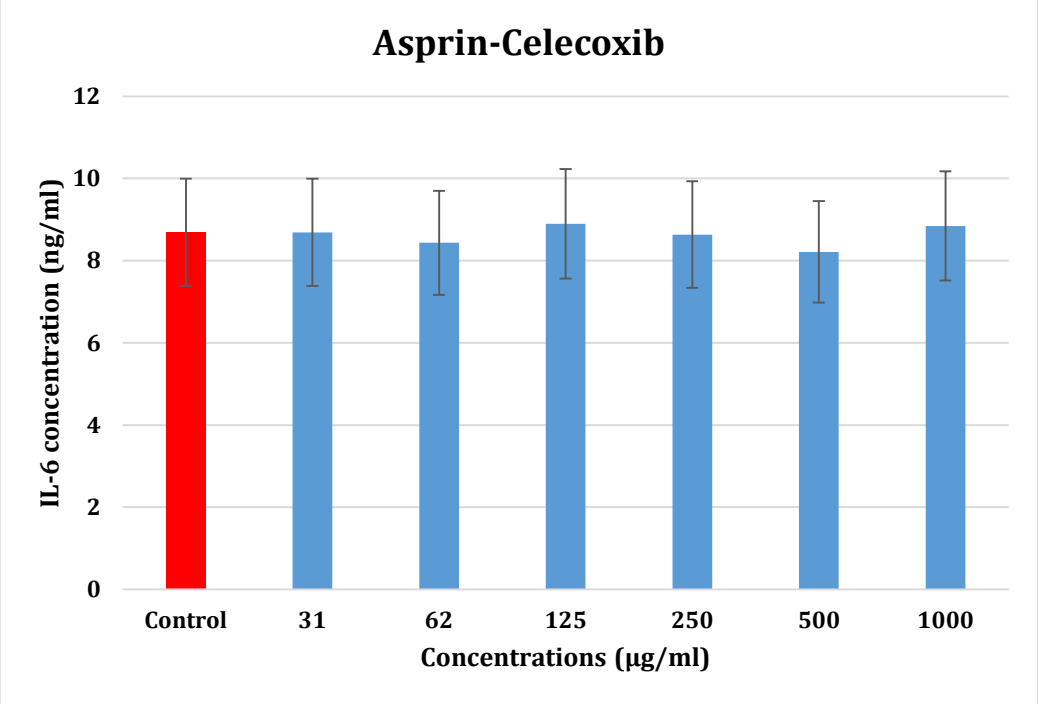


Figure (3.16): Effect of (Aspirin – Celecoxib) combinations at equal concentrations on IL-6.

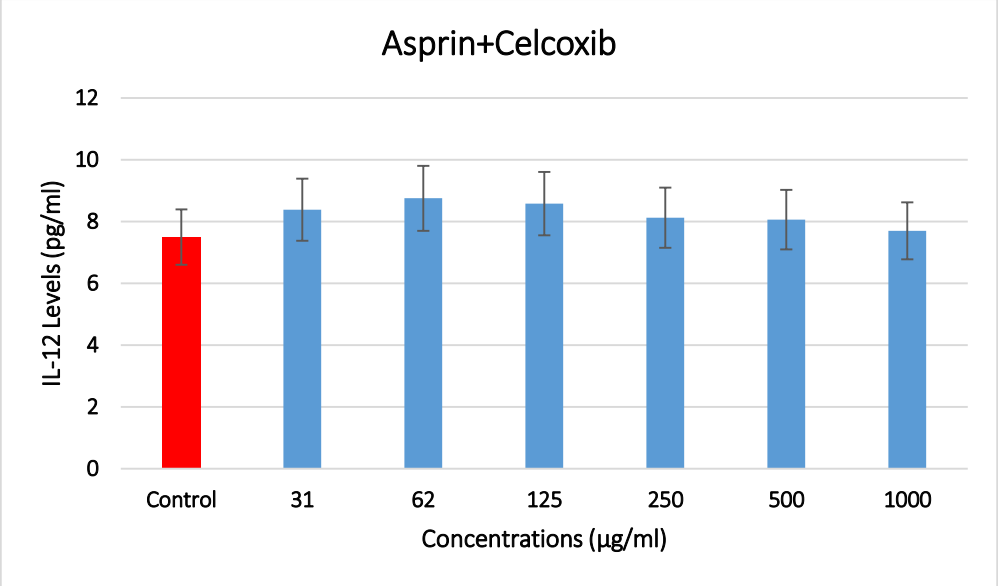


Figure (3.17) Effect of (Aspirin – Celecoxib) combinations at equal concentrations on IL-12.

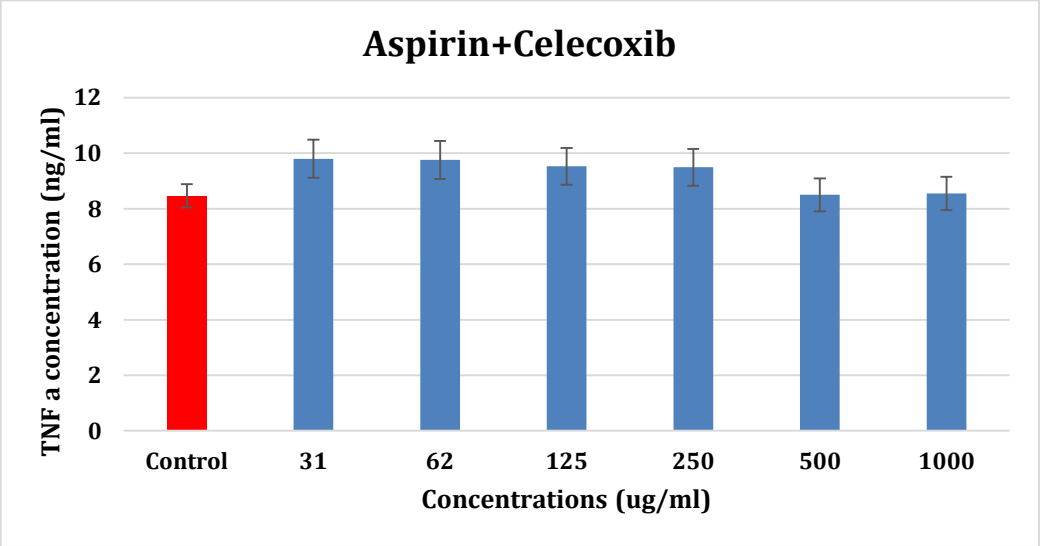


Figure (3.18): Effect of (Aspirin – Celecoxib) combinations at the equal concentration on (TNF-α).

3.11 Effect of Aspirin - Sulindac combinations on cytokine production.

The result showed that there was a statistically significant increase when used (Aspirin- sulindac) combination treatment on the production of (TNF-α**)** compared to the control group as shown in figure (3.21), while there was no statistically significant effect of the (Aspirin-Sulindac) combination treatment on the production of IL6 and IL12 compared to the control group as shown in figure (3.19) and (3.20) respectively and as shown in table (3-6) (3-7) and (3-8) respectively.

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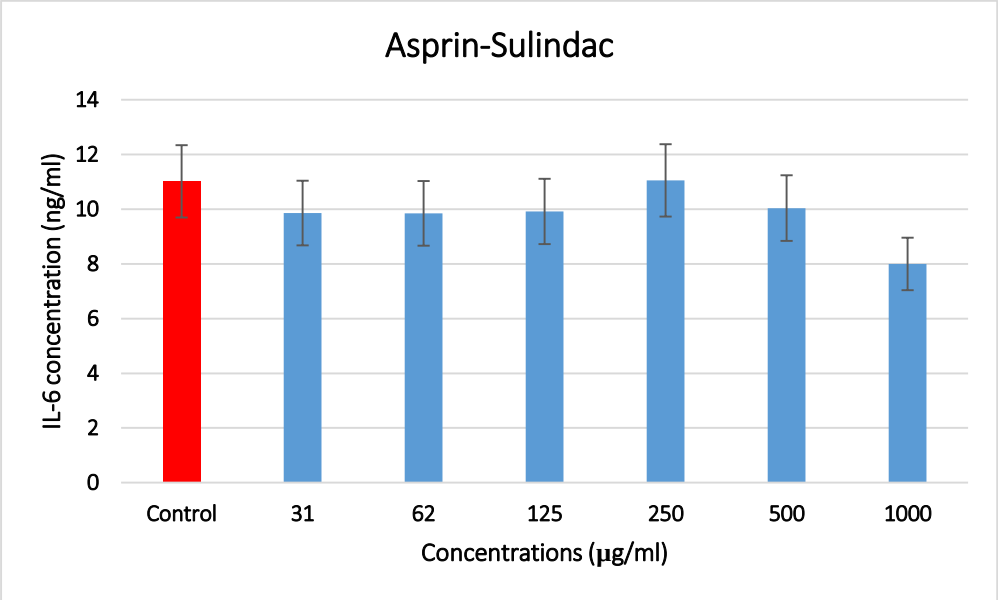

Figure (3.19): Effect of (Aspirin – Sulindac) combination at the equal concentration on IL-6.

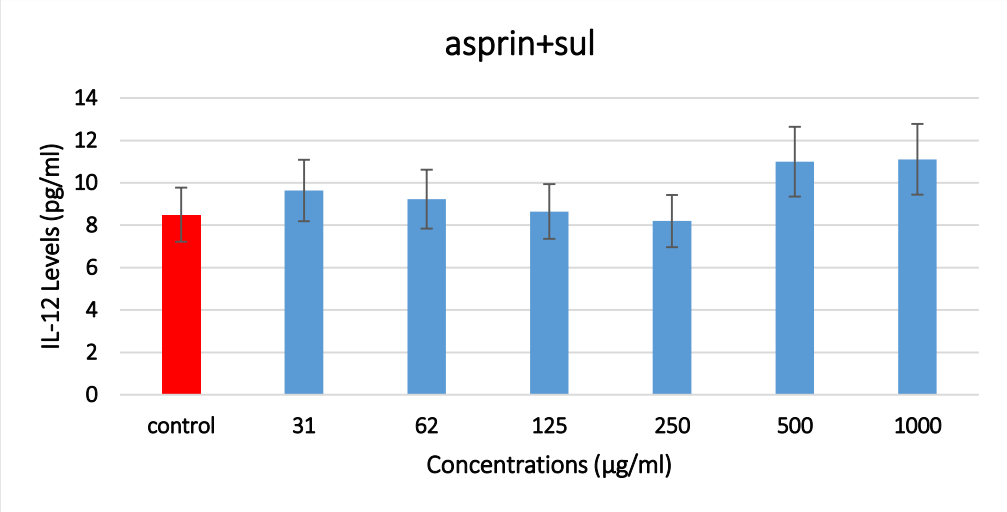

Figure (3.20): Effect of (Aspirin – Sulindac) combination at the equal concentration on IL-12.

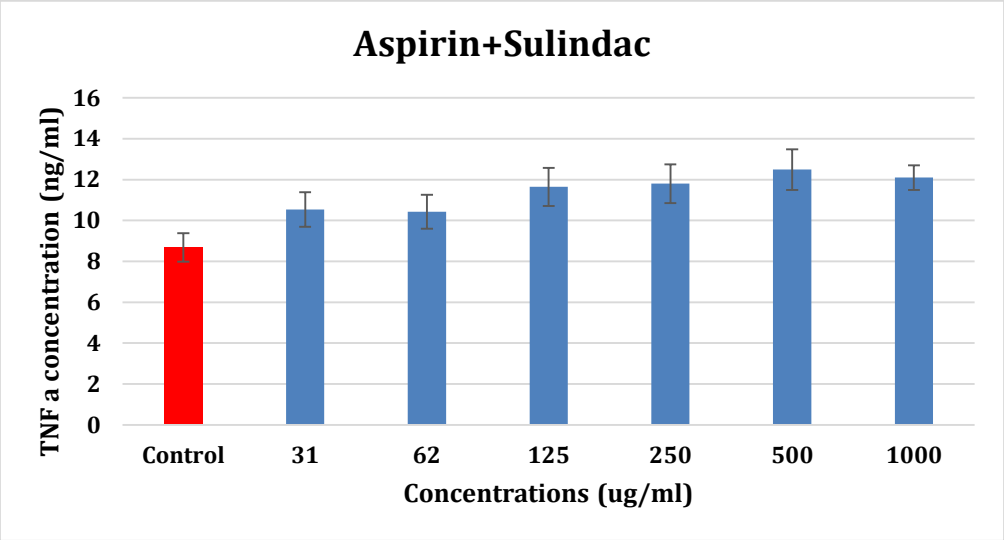

Figure (3.21) Effect of (Aspirin – Sulindac) combination at the equal concentration on TNF-α.

3.12 Effect of Celecoxib - Sulindac combinations on cytokine production.

The result showed that there was no statistically significant effect of the Celecoxib - Sulindac combinations treatment on the production of IL-6 and TNF- α to the control group as shown in figure (3.22) and (3.24) respectively, while there was a statistically significant increase of the Celecoxib - Sulindac combinations treatment for concentration (125,250,1000 µg/ml) and no statistically significant effect for (31,62,500 µg/ml) on the production of IL-12 as shown in figure (3.23) and as shown in table (3-6) (3-7) and (3-8) respectively.

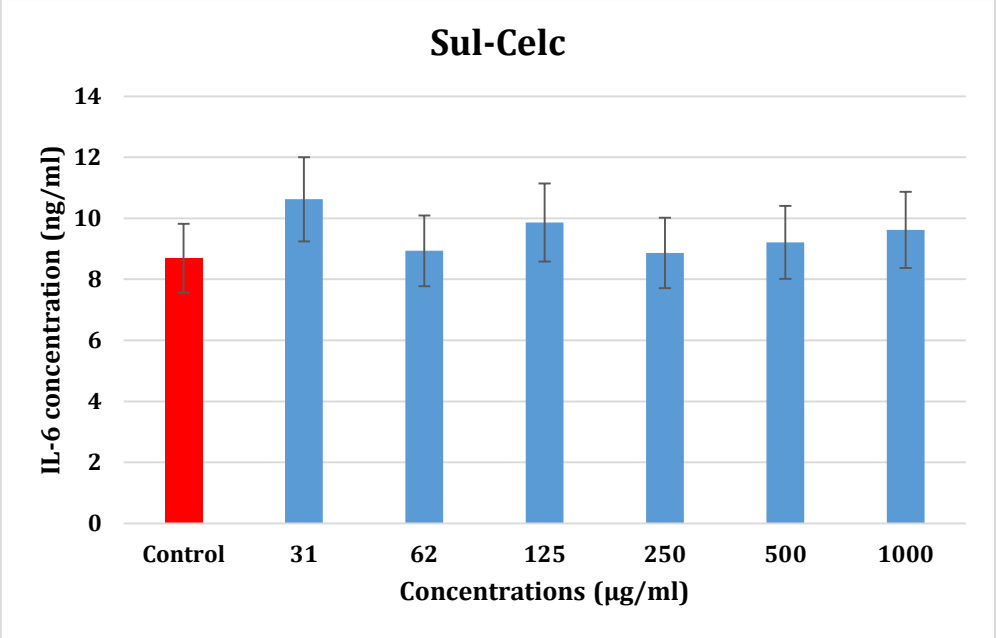

Figure (3.22): Effect of (Celecoxib -Sulindac) combination at the equal concentration on IL-6.

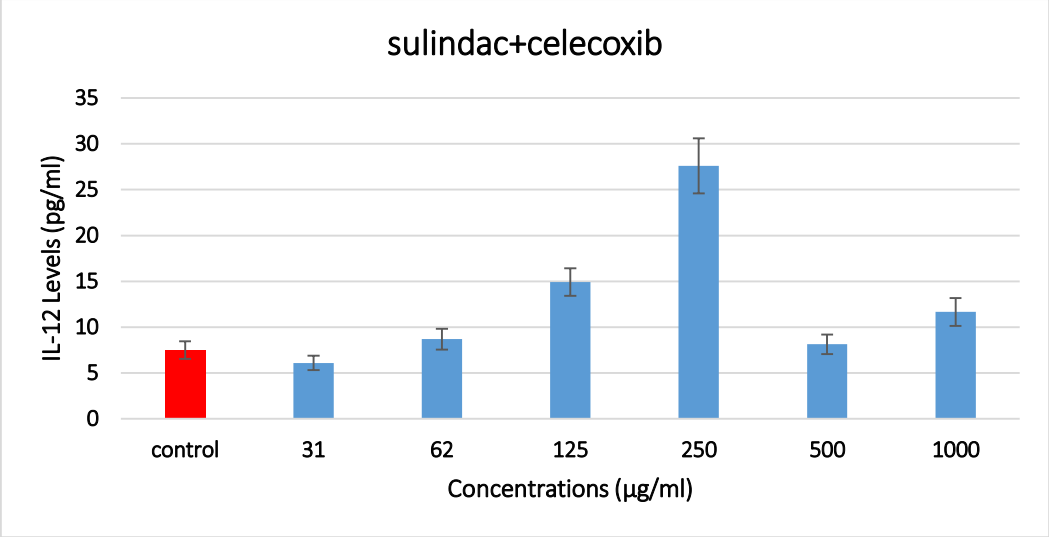

Figure (3.23): Effect of (Celecoxib -Sulindac) combination at the equal concentration on IL-12.

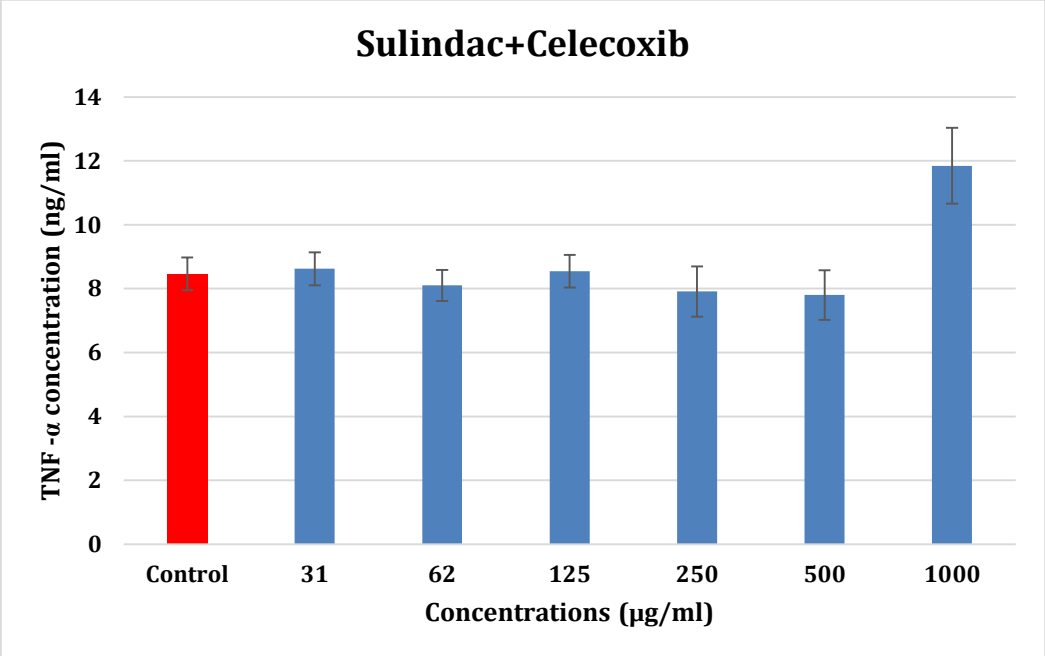

Figure (3.24): Effect of (Celecoxib -Sulindac) combination at equal concentration TNF-α.

Chapter Four

Discussion

4. Discussion

4.1 Effect of Aspirin on the viability of colon cancer cells line.

Our result showed that the concentration of (31µg/ml) of aspirin caused a significant cytotoxic effect while showed a non-significant change in cells proliferation at concentrations of (125 and 250) µg/ml as shown in figure (3.1). In (Deb et al., 2011) study 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 stage of the cell cycle, because aspirin inhibits the nuclear factor kappa B (NF-B) pathway.

Aspirin inhibits cancer in two ways COX dependent or COX-independent mechanism. Aspirin can inhibit the COX-2-dependent generation of aspirin-triggered lipoxins, which inhibit cell proliferation and attenuate COX-2-mediated activation of carcinogens. Aspirin had direct modulation of oncogene-induced expression of transcription factors, such as nuclear factor kappa B (NFκB) which cause increased apoptosis of cancer cells (Manuscript, 2012).

Many evidence shows that aspirin at 5 mM concentration has very different effects on cells than at lower concentrations, 50 and 500 mM, a finding that supports clinical evidence that aspirin at low and high doses has different effects. Aspirin predominantly induces genes involved with DNA damage signaling, nucleotide metabolism, and the stress response. Rac1 induction by aspirin may underlie the beneficial effects of aspirin in colon cancer progression (Hardwick et al., 2004).

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)

A more recent analysis of data from two large randomized trials with aspirin concluded that a daily dose of 300 mg or more of aspirin taken for 1 – 7 years could prevent colorectal cancer after a latency period of about 10 years. The study raises the possibility that NSAIDs taken 4 – 7 days per week might be sufficient to prevent adenomas and, by implication, colorectal cancer. (Grau et al., 2009)

When it comes to the toxicity of aspirin on colorectal cancer cells, a number of molecular pathways have been implicated. Aspirin, for example, has been shown to upregulate NF-B expression selectively in colorectal cells, which has been linked to apoptosis.Aspirin inhibits the expression of NF-B. (Deb et al., 2011)

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)

Other investigators who have tested aspirin on colon cancer cells have also reported requirements of 5–10 mM for at least 50% inhibition of cell growth. Although aspirin is recommended for chemoprevention of colon cancer in men. In conclusion Aspirin_ Phosphatidylcholine exerts a growth inhibition to colon cancer cells that is more efficacious than the NSAID alone. Aspirin_ Phosphatidylcholine inhibits DNA synthesis and proliferation of colon cancer cells (Dial et al., 2006).

4.2 Effect of sulindac on the viability of colon cancer cell line

Our results showed a highly significant decrease in cells viability for all concentrations of sulindac as shown in Figure (3.2). These findings are close to the result of (Piazza et al., 2009b) that said Sulindac is regarded as one of the most effective NSAIDs due to its potential to cause colonic adenomas to regress in people with familial adenomatous polyposis (FAP). Its advantages are restricted to a 60% to 70% reduction in the number and size of polyps. Several researchers have determined that the mechanism behind NSAIDs' tumor cell growth inhibitory action may be COX independent in part or whole.The ability to suppress DNA synthesis and induce apoptosis has been linked to sulindac sulfide (SS) and sulindac sulfide amide (SSA).

Nuclear b-catenin in vivo, indicating a direct relationship between sulindac's tumor-suppressive actions and a fundamental colorectal cancer flaw, namely, unregulated Wnt-signaling. Colorectal cancer is initiated by mutations involving components of the Wnt-signaling cascade, notably in b-catenin. Total b-catenin levels were only moderately reduced after treatment with sulindac sulfide(Boon et al., 2004).

Sulindac sulfide can selectively inhibit colon cancer cell proliferation and induce apoptosis by inhibiting PDE5 to increase intracellular cGMP levels and activate PKG signaling. The relevance of this pathway to tumorigenesis was shown by its ability to inhibit Wnt/ β-catenin-dependent T-cell factor transcriptional activity, which leads to the suppression of critical proteins that regulate colon tumor cell proliferation and apoptosis(Li et al., 2013).

(Rahman et al., 2000) reported that Sulindac had a significant inhibitory effect at 100 mM in hepatocellular carcinoma (HCC) cell lines, whereas in pancreatic and colon cancer cell lines it was 200 and 400 mM,

respectively. The anti-proliferative effect or induction of apoptosis has been referred to as this antineoplastic effect. Both cell cycle arrest and apoptosis contributed to the growth-inhibitory effect of sulindac in HCC cell lines (Rahman et al., 2000).

Sulindac's capacity to selectively suppress cancer cell proliferation and cause apoptosis without damage to normal proliferating tissues, as is the case with traditional chemotherapy, may be extremely relevant to their safety and efficacy for cancer chemoprevention. (Li et al., 2013).

4.3 Effect of Celecoxib on the viability colon cancer cell line .

The result showed that there was a highly significant decrease in cells viability for all concentrations used as shown in Figure (3.3). 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).

According to the (Reddy et al., 2006) study that when compared with individual high doses of atorvastatin and celecoxib, combinations of these agents at low doses induce greater inhibition of colon carcinogenesis. The results of this study indicate that celecoxib and atorvastatin are given individually at high doses and in combination at low doses showed no evidence of gastrointestinal ulceration and bleeding, suggesting an absence of any side effects.

Celecoxib can induce severe oxidative stress, mitochondrial redox homeostasis, and promotes colon cancer cell apoptosis (Z. Zhang et al., 2018)

(Wen et al., 2020) Said that celecoxib can inhibit the transduction of the Wnt/ signaling pathway through COX-2 dependent and non-dependent mechanisms which inhibit colon cancer. Celecoxib improves

the chemotherapy effect of gefitinib on non-small cell lung cancer by inducing cancer cell apoptosis by inhibiting hepatocyte growth factor receptor, HGFR)/T-lymphokine-activated killer cell-originated protein kinase (TOPK) signaling pathway. Celecoxib can treat refractory lymphoid malformations by inhibiting the mechanism of COX-2/PGE2.

Celecoxib has shown 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 tumors but also induce apoptosis of tumor cells. Furthermore, its inhibition of cancer cells appears to be time and concentration-dependent (Hu et al., 2020).

4.4 Effect of combination of Aspirin and Sulindac on the viability of colon cancer cells line.

The result showed a highly significant decrease in cell viability for the concentrations (1000,500,250 µg/ml), while other concentrations (125,62.5 and 31.25) showed a non-significant increase in cell viability in comparison with the control group after incubation for 24 hrs, as shown in Figure (3.4), That result close to(Wang et al., 2022)

The study said COX-2-independent effects of low-dose of NSAIDs on colorectal cancer cell motility and Store-operated Ca2+ channel (SOC) activity. Two distinct SOC inhibitory mechanisms for Aspirin and Sulindac. First, Sulindac suppresses stromal interaction molecule 1(STIM1) translocation, and second, Aspirin obstructs the STIM1-Orai1 (Ca2+ release-activated calcium channel protein 1 known as Orai1) interaction. Taken together, the current results demonstrate a novel mechanism by which NSAIDs may affect CRC metastasis. Aspirin may be especially useful as a SOC inhibitor in clinical settings.

4.5 Effect of Aspirin - Celecoxib combinations on the viability of SW colon cancer cells line.

our result showed a highly significant decrease in cell viability for all concentrations used after incubation for 24hrs with the drug in comparison with the control group, as shown in Figure (3.5).

This may be due to 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).

According to (Ekanem et al., 2020) When using low doses of atorvastatin and celecoxib, and /or aspirin in combinations of these agents greater inhibition of colon carcinogenesis than high doses. This combination at low doses showed no evidence of gastrointestinal ulceration and bleeding, suggesting an absence of any side effects (Ekanem et al., 2020).

4.6 Effect of Sulindac - Celecoxib combinations on the viability of SW colon cancer cells line

Our result showed a highly significant decrease in cell viability for all concentrations after incubation for 24hrs in comparison with the control group, as shown in Figure (3.6)

(Vaish & Sanyal, 2012) said that Sulindac and Celecoxib might regulate Na+/H+ intracellular concentration for chemoprevention of

colorectal cancer. Sulindac and Celecoxib treatment has increased intracellular calcium levels as compared to DMH. Higher [Ca2+] I have been observed to be involved in the initiation of cell death via apoptosis Therefore, the down-regulation of Na-H exchanger isoform 1 (NHE-1) and an increase in [Ca2+] I in NSAIDs co-administered groups may help in reducing pHi and reducing the caner load. Bcl-2 down-regulation by NSAIDs suggests that Sulindac and Celecoxib can initiate the intrinsic pathway of apoptosis.

Many studies have reported the synergistic effect of COX inhibitors for the sensitivity to anticancer drugs. Celecoxib induced apoptosis in cultured head and neck cancer cell lines (Surgery, 2003).

Because inducible isoforms of nitric oxide synthase and COX-2 appear to be involved in the pathogenesis of colon cancer, selective inhibitors that reduce their activities may be potential chemopreventive agents. Co-administration of an inducible isoform of nitric oxide synthase inhibitor and a COX-2(celecoxib or sulindac) inhibitor in the diet significantly suppresses AOM-induced colonic ACF formation (Rao et al., 2002).

Several studies have recorded the effect of COX inhibitor (sulindac and celecoxib) for chemoprevention of colorectal cancer and initiate apoptosis and many studies have reported the synergistic effect of COX inhibitors for the sensitivity to anticancer drugs there for we mixed up sulindac with celecoxib to give more potent effect than use alone.

4.7 Effect of Aspirin treatment on the cytokines production.

Our 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 figure (3.7) (3.8) and (3.9) respectively. But several studies have shown aspirin decrease

IL-6 this is possible due to using a low dose of aspirin and aspirin's effect on IL-6 needs a high dose.

(Bergman et al., 2011)found that interaction between stimulated peripheral blood mononuclear cells PBMC and HT-29 cells in cytokines IL-6. And found that aspirin inhibited the production of IL-6 and IL-11 by cells of four cancer lines. The study conclude that separate incubation of either non-stimulated PBMC or cells of the two colon cancer lines with aspirin did not appear any significant effect on cytokine generation, except for IL-6 produced by PBMC (Bergman et al., 2011).

(Slattery et al., 2007) suggest associations between IL6 genotypes and CRC risk are modified by aspirin/NSAIDs. Use of aspirin/NSAIDs: especially for colon cancer, users had a greater reduction in risk if they also had a C allele in either IL6 polymorphism. Interaction between VDR genotype and IL6 also was influenced by the use of aspirin/NSAIDs.

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

Until understanding the effect of aspirin on anticancer mechanism must take into account optimal dose, frequency of administration, and combination therapy with other agents. (Manuscript, 2012)

4.8 Effect of Celecoxib drug on cytokine production.

Our result showed that was a statistically significant decrease when using Celecoxib drug for concentration (500, 1000 µg/ml) on IL-6, while there was no statistically significant effect of the Celecoxib treatment on the production of IL-6 for concentration (31,62,125,250 µg/ml), IL-12

and TNF- α for all concentration in compared to the control group as shown in figure (3.10),(3.11) and (3.12) respectively. That agrees with (Y. Liu et al., 2011) who said that 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.

4.9 Effect of Sulindac drug on cytokine production.

The result showed that there was no statistically significant effect of the Sulindac treatment on the production of IL-6 and IL-12.

While there was a statistically significant increase for concentration 1000µg/ml when used sulindac drug on the production of IL-12 and in used Sulindac drug on the production of TNF-α for concentration (500,1000µg/ml) in comparison with the control group as shown in figure (3.13), (3.14) and (3.15) respectively

These findings are closed to the result of (Berman et al., 2002) that said TNF-α either alone or in combination with sulindac induces apoptosis of lung cancer cells. But TNF-α or sulindac alone had little effect on apoptosis, while the combination of these agents can increase apoptosis of lung cancer cells. The influence of TNF-α and sulindac suggest that sulindac inhibition of NF-κB may serve as an effective therapy in cancer. The inhibition of the NF-κB pathway leads to the

increased apoptosis observed in a carcinoma cell line in response to treatment with TNF-α in combination with sulindac.

Also (Yasui et al., 2003) reported that sulindac strongly inhibited TNF-α–mediated NF-κB activation. Specifically inhibits NF-_κB–mediated transcription. Therefore the combination of TNF-α and sulindac enhances TNF-α–mediated apoptosis by inhibiting NF-_κB activation in two non-small cell lung carcinoma cell lines in vitro. The study demonstrates that frequent intratumor injection of sulindac with TNF-α more strongly reduces subcutaneous tumor progression of gastric carcinoma cells than either sulindac or TNF-α alone.

4.10 Effect of Aspirin - Celecoxib combinations on cytokine production.

The result showed that there was no statistically significant effect (P>0.001) of the Aspirin- Celecoxib combination treatment on the production of IL-6, IL-12. While there was a statistically significant increase for concentration (31, 62 µg/ml) and no statistically

Significant effect for concentration (125,250,500.1000µg/ml) of Aspirin - Celecoxib combinations on the production of TNF-α compared to the control group as shown in figure (3.16) (3.17) and (3.18) respectively. That result may be because we use a low dose of aspirin as reported in (Ruzov et al., 2016) which said celecoxib interacts with the COX-1 enzyme and can potentially interfere with its inhibition by aspirin.

The interaction between them is mechanism-based pharmacokinetic _ pharmacodynamic (PK-PD) modeling to analyze the interaction of these drugs. The modeling results indicate that celecoxib can attenuate to a limited extent the in vivo antiplatelet effects of aspirin.

Celecoxib and aspirin reduce IL-6 when using each drug alone but in combination, there is no effect because of drug-drug interaction between them.

Our result has seen a TNF-α increase that agrees with (Page et al., 2010) that said an increase in TNF back to that it is the NSAID-induced reduction in PGE2 level. The expression increase of the key pro-inflammatory cytokine TNF in rheumatoid synovia, in isolated peripheral blood monocyte populations, and at the systemic level in whole blood.

4.11 Effect of Aspirin - Sulindac combinations on cytokine production.

The result showed that there was a statistically significant increase when used (Aspirin- sulindac) combination treatment on the production of (TNF-α **)** α compared to the control group as shown in figure (3.21), while there was no statistically significant effect (P>0.001) of the (Aspirin- Sulindac) combination treatment on the production of IL6 and IL12 a compared to the control group as shown in figure (3.19) and (3.20). This may be due to the effect of sulindac on TNF-α as noted in (Zou et al., 2009) said Sulindac and other NSAIDs enhanced.

TNF-α release from LPS pretreated, macrophage-derived RAW264.7 cells at concentrations achieved clinically in humans. Sulindac is a potent inhibitor of the NF-κB pathway through inhibition of Iκ kinase activity. As known NF-κB plays an important role in preventing TNF-α-induced cell death. As a result, sulindac may sensitize hepatocytes to TNF-_-induced cell death through inhibition of NF-_B pro-survival signaling(Zou et al., 2009).

In our result, Aspirin is not affected on IL-6 even in combination with sulindac may be due to needing high doses of Aspirin to work.

4.12 Effect of Celecoxib - Sulindac combinations on cytokine production.

Our result showed a significant increase in TNF-α that may back up to sulindac effect as we discussed that in sulindac alone. And our result showed an increase in IL-12 that may back up to the celecoxib effect when used in a low dose that agrees with (Stolina et al., 2000) who said that COX-2 inhibitor drugs result in up-regulation of IL-12 level. Increased level of IL-12 may promote increased type 1 cytokine release and thus enhanced cell-mediated antitumor immune responses.

Celecoxib when used at high concentration blocks the secretion of the αβ and β2 dimeric forms of IL-12 by a mechanism that is independent of COX2 inhibition. Endoplasmic reticulum ER factors may be involved in the blockage of IL-12 production when cells are treated with celecoxib (Alloza et al., 2006). When we combined these drag results synergistic effect that is important in inhibition of tumor cell.

Conclusions and Recommendations

Conclusions

From our research we conclude:

1. Aspirin drug showed no significant change to the IL-6 level this is possible due to low doses used because it needs high dose as proven by other research.
2. Celecoxib drug showed a significant decrease in IL-6 level when use alone and showed a significant decrease in IL-12 at high concentration in combination with sulindac compared to the control group.
3. Combination of (Aspirin –Sulindac), (Aspirin –Celecoxib), and (Sulindac-Celecoxib) has a better significant cytotoxic effect than each drug alone on SW480 colon cancer cell line.
4. Nonsteroid anti-inflammatory drugs NSAIDs (Aspirin, Sulindac, Celecoxib**)** have a cytotoxic effect on SW480 colon cancer cell line.
5. Sulindac drug showed a significant increase in TNF-α level when used alone or in combination with aspirin compared to the control group.

Recommendations

1. Investigate the anti-proliferative and anticancer effect of NSAID on other types of the cancer cell line.
2. Study the immunomodulatory effect of NSAIDs on animal models.
3. Study the effect of NSAIDs on other types of cytokine.
4. Study the action of NSAIDs in combination with anticancer drugs because of a synergistic effect of it.

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ملخص

سرطان القولون والمستقيم (CRC) هو أحد الأورام الرائدة في العالم ويعتبر من أكبر الأمراض القاتلة ، إلى جانب سرطان الرئة والبروستاتا والثدي. يُعرف سرطان القولون والمستقيم أيضًا بسرطان القولون والمستقيم الغدي. هناك خطر كبير من CRC في الأفراد الذين لم تتم إزالة الأورام الغدية ، واستئصال السليلة يقلل من خطر CRC. يظهر سرطان القولون والمستقيم (CRC) اختلافات في الإصابة ، والتسبب في المرض ، والمسارات الجزيئية ، والنتيجة اعتمادًا على موقع الورم. قد يشمل العلاج الجراحة والعلاج الإشعاعي والعلاج الكيميائي التي ترتبط غالبًا بالعديد من الآثار الجانبية والسمية والمكلفة ، مما يؤثر بشكل كبير على جودة حياة المرضى. العقاقير غير الستيرويدية المضادة للالتهابات (NSAIDs) هي مثبطات COX المعروفة ، فهي حتماً مرشح شائع مضاد للسرطان في علاج السرطان والوقاية منه. استخدام مضادات الالتهاب غير الستيروئيدية في سرطان القولون والمستقيم لأنها تفرز ويمكن استخدامها كوسيلة وقائية لـــ CRC ويمكن استخدامها مع أدوية السرطان الأخرى.

الغرض من هذه الدراسة هو التحقيق في التأثير السام للخلايا للأدوية غير الستيرويدية المضادة للالتهابات (الأسبرين ، والسولينداك ، والسيليكوكسيب) على خط خلايا سرطان القولون والمستقيم SW480.

تم تنفيذ العمل العملي للدراسة البحثية في معمل أبحاث طلاب الدراسات العليا / قسم علم العقاقير والسموم / كلية الطب / جامعة بابل

تم الكشف عن التأثيرات المناعية لمضادات الالتهاب غير الستيروئيدية عن طريق قياس إنتاج السيتوكينات في طاف خلايا سرطان القولون والمستقيم استجابةً لمضادات الالتهاب غير الستيروئيدية.

تضمنت تجربة أربعة أعمدة من ستة مكررات من 96 طبقًا جيدًا تم زرعها بخطوط خلايا سرطان القولون SW480 بتركيز 5 * 105 خلية. تم اعتبار العمود رقم 1 الذي يحتوي على ستة مكررات كمجموعة ضابطة ، وتم تعريض الأعمدة الثلاثة اللاحقة لكل عقار (Aspirin و Sulindac و Celecoxib) بتخفيف متسلسل قدره (1000 ، 500 ، 250 ، 125 ، 62.5 ، 31 ميكروغرام / مل)) ، أربعة مكررات لكل تركيز. حضنت مرة واحدة لمدة 24 ساعة. تأثير (الأسبرين ، تم تقييم Sulindac و celecoxib) على نمو خط سرطان القولون بواسطة اختبار MTT.

كما في العمل أعلاه ، لكن التعرض لمزيج من مضادات الالتهاب غير الستيروئيدية (سولينداك – أسبرين ، سيليكوكسيب – سولينداك ، أسبرين – سيليكوكسيب). تم تقييم تأثير هذا المزيج على نمو خط سرطان القولون بواسطة مقايسة MTT.

أظهرت نتائج السمية الخلوية انخفاضًا معنويًا (5P <0.0) في قابلية بقاء SW 480 لجميع تركيزات السولينداك والسيليكوكسيب والسولينداك والسيليكوكسيب والأسبرين والسيليكوكسيب مقارنة بالمجموعة الضابطة .

أظهرت نتائج السمية الخلوية انخفاضًا معنويًا (5P <0.0) في قابلية بقاء SW 480 للتركيز (31 ميكروغرام / مل) من الأسبرين والتركيزات (1000.500.250 ميكروجرام / مل) من الأسبرين– سولينداك مقارنة بالمجموعة الضابطة.

تم إجراء اختبار ELISA للكشف عن مستويات 6-Interleukin و nterleukini-12 وعامل نخر الورم ألفا. أظهرت النتائج وجود انخفاض معنوي (0.0> 5P) عند استخدام عقار Celecoxib للتركيز (500 ، 1000 ميكروغرام / مل) على IL-6.

أظهرت نتائجنا زيادة ذات دلالة إحصائية في التركيز 1000 ميكروغرام / مل عند استخدام عقار السوليندك على إنتاج IL-12 وعلى إنتاج α-TNF للتركيز (500 ، 1000 ميكروغرام / مل) مقارنة بمجموعة التحكم.

أظهرت النتائج وجود زيادة ذات دلالة إحصائية (0.0> 5p) عند استخدام العلاج المركب (sulindac -Aspirin) على إنتاج (α-TNF) α مقارنة بمجموعة التحكم. بينما عالجت تركيبات (سيليكوكسيب - سوليندك) زيادة ذات دلالة إحصائية (0.0> 5P) للتركيز (125،250،1000 ميكروغرام / مل) على IL-12.

أظهرت النتائج تأثيرات مضادة للتكاثر العقاقير غير الستيرويدية المضادة للالتهابات (NSAIDs) على خط خلايا سرطان القولون والمستقيم عند استخدام كل دواء بمفرده أو مع تأثيرات تعديل المناعة على إنتاج السيتوكينات.

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جامـــعة بابـل
كليـة الطب

تأثير الاسبرين والسوليندك والسيلكوكسب ومزجهاعلى حيويه الخليه ومستوى السيتوكين في خط خلايا سرطان القولون والمستقيم SW480

رسالة

مقدمة الى مجلس كلية الطب جامعة بابل استيفاء جزئي لشروط الحصول على درجة الماجستير في ألادويه/ ادويه وسموم

من قبل

رسل كاظم بخيت

بكلوريوس الصيدلة

بأشراف

أ.م ماجد كاظم عباس

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