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MASTER'S DEGREE REPORT

Topic

Study of hematological toxicity and oxidative stress induced by anticancer drugs

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Dedications

I dedicate this work:

To my precious parents **Nadia** and **Djamal-Eddine** for working hard to make me the girl I am, for their endless encouragements and their unconditional love.

To my lovely sisters **Yasmine** and **Saloua** for their help, complecity, love, presence and lightheartedness.

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With all of my love,

Lyna.B

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4HNE: 1/4-hydroxy-2-nonenal.

5FU: 5-Fluouracil.

5FUTP: 5-Fluouracil-3-phosphate.

ABTS: 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid.

ADP: Adenosine diphosphate.

AIF: Apoptosis-Inducing Factor.

AIFM2: Mitochondrial apoptotic inducing factor-2.

APAF: Apoptotic Protease Activating Factor.

ATM: Ataxia telangiectasia mutated.

ATP: Adenosine triphosphate.

ATP5B: ATP synthase subunit.

ATR: Ataxia telangiectasia and Rad3-related kinase.

BAK: BCL2 Antagonist/Killer.

BAX: BCL-2-associated X protein.

BCC: Blood cells count.

BCL2: B-Cell lymphoma 2.

BCL-xl: B-cell lymphoma-extra-large.

CASP8: Caspase 8.

CDC25 A: Cell Division Cycle 25 phosphatase A.

CDCD25 C: Cell Division Cycle 25 phosphatase C.

CDK: Cycline dependant kinase.

CHK1: Checkpoint 1.

CHK2: Checkpoint 2.

CPR: Cytochrome P450 reductase.

CYP: Cyclophosphamid.

DLD: Dihydrolipoamide dehydrogenase.

DNA: Deoxyribonucleic acid.

DNR: Daunorubicin.

DOX: Doxorubicin.

DR5: Death Receptor 5.

dUMP: Deoxyuridine monophosphate.

EDTA: Ethylene diamine tera acetic acid.

EPI: Epirubicin.

FADH2: Flavin adenine dinucleotide.

FAS: Fas cell surface death receptor.

FASL: Fas Ligand.

GATA4: GATA binding protein 4.

G-CSF: Granulocyte colony stimulating factors.

GPX: Glutathione peroxidase.

GSH: Glutathione.

H2O2: Hydrogen peroxide.

HGB: Hemoglobin.

L-OHP: Oxaliplatin.

MDA: Malondialdehyde.

MetHb: Methemoglobin.

NaCl: Sodium chloride.

NADH: Nicotinamide adenine dinucleotide.

NADPH: Nicotinamide adenine dinucleotide phosphate.

NaOH: Sodium hydroxide.

ND: NADH-dehydrogenase.

NOXA: Phorbol-12-myristate-13-acetate-induced protein 1.

OS: Oxidative stress.

P16: Cyclin-Dependent Kinase Inhibitor 2A.

P38: P38 Mitogen-Activated Protein Kinase.

P53: Tumor protein p53.

PLT: Platelets.

PUMA: P53 Upregulated Modulator of Apoptosis.

RBC: Red blood cells.

RNA: Ribonucleic acid.

RNS: Reactive nitrogen species.

ROS: Reactive oxygen species.

SDHA: Succinate dehydrogenase A.

SMAC/DIABLO: Second Mitochondria-derived Activator of Caspases/Direct IAP-binding protein with Low pl.

SOD: Superoxide dismutase.

TAC: Total antioxidant capacity.

TBA: Thiobarbituric acid.

TBARs: Thiobarbituric acid reactive substances.

TCA: Trichloroacetic acid.

TNF: Tumor necrosis Factor.

TNFR1: Tumor Necrosis Factor Receptor 1.

TOP1: Topoisomerase 1.

TOP2: Topoisomerase 2.

TRAP: Total antyoxidant capacity of plasma.

TS : Thymidilate Synthase.

TXT : Docetaxel.

UV : Ultraviolet.

WBC: White blood cells.

Introduction

Introduction

Cancer is a challenging health condition worldwide and is classified as the second leading cause of death among human diseases. Although several recent anticancer drugs have been developed and clinically implemented, chemotherapy remains the primary treatment for several types of cancer; it systematically stops cell proliferation, prevents the development of metastases, and causes tumor shrinkage **(Chen et al., 2022; Mameri et al., 2021).** However, due to its cytotoxic nature, chemotherapy damages both healthy and cancer cells, leading to numerous side effects, including cardiotoxicity, gastrointestinal disorders, nephrotoxicity, and hematotoxicity **(Lowenthal and Eaton, 1996; Testart-Paillet et al., 2007).**

Hematological toxicity is the result of the myelosuppression caused by nearly all chemotherapy agents or the direct effect of the administered drug **(Lowenthal and Eaton, 1996; Testart-Paillet et al., 2007).**

The mechanism of action of anti cancer drugs involves the production of reactive oxygen species (ROS). The high production of ROS is characterized by methemoglobin formation, the elevation of lipid peroxidation products, and the diminution of the total radical-trapping capacity of blood plasma; it also disturbs the cell's homeostasis, structure, and function, including membrane integrity, especially in red blood cells **(Conklin, 2004; Mameri et al., 2021).**

Evidence in the literature indicates that anticancer drugs induce red blood cell lysis through direct contact, that involves an oxidative process and changes in antioxidant defenses **(Mameri et al., 2021; Panis et al., 2012).** To our knowledge, the direct effect of chemotherapy on whole blood cells is still under investigation.

This study aims to evaluate the direct hematotoxic effect of chemotherapy in cancer patients by evaluating both quantitative and qualitative parameters before and after the treatment administration, as well as the underlying mechanisms.

This manuscript includes four chapters: the first two chapters contain a general review about chemotherapy, oxidative stress, and the link between chemotherapy and oxidative stress. The third chapter describes the materials and methods used throughout the experimentation. The fourth chapter highlights the results as well as their interpretation, and finally, it ends with a conclusion and further perspectives.

Chapter I

Chemotherapy

I. Chemotherapy

I.1. Definition

Chemotherapy is a therapeutic strategy commonly used for cancer, often combined with surgery and radiotherapy. It uses cytotoxic drugs resulting in a total elimination of some type of cancer or shrinking tumors in others **(MacDonald, 2009).**

I.2. Chemotherapy drug classification

I.2.1. Alkylating agents

Alkylating agents are a chemotherapy drug class that includes nitrogen mustards, ethyleneimine, nitrosoureas, alkyl sulfonate, platinum-based compounds, and non-classical compounds. They are widely used to treat several types of cancer, such as glioma, lymphoma, ovarian neoplasm, and lung and bladder tumors **(Lang et al., 2023).** They directly interact with DNA throughout the entire cell cycle. This interaction leads to DNA crosslinking, resulting in DNA strand breaks, mispairing, and inhibition of cell division, which can potentially induce cell death **(Figure 01).** Alkylating agents can be monofunctional, by interacting with one DNA strand, or bifunctional, by reacting with an atom of each strand, which results in a covalent crosslink **(Ralhan and Kaur, 2007).**

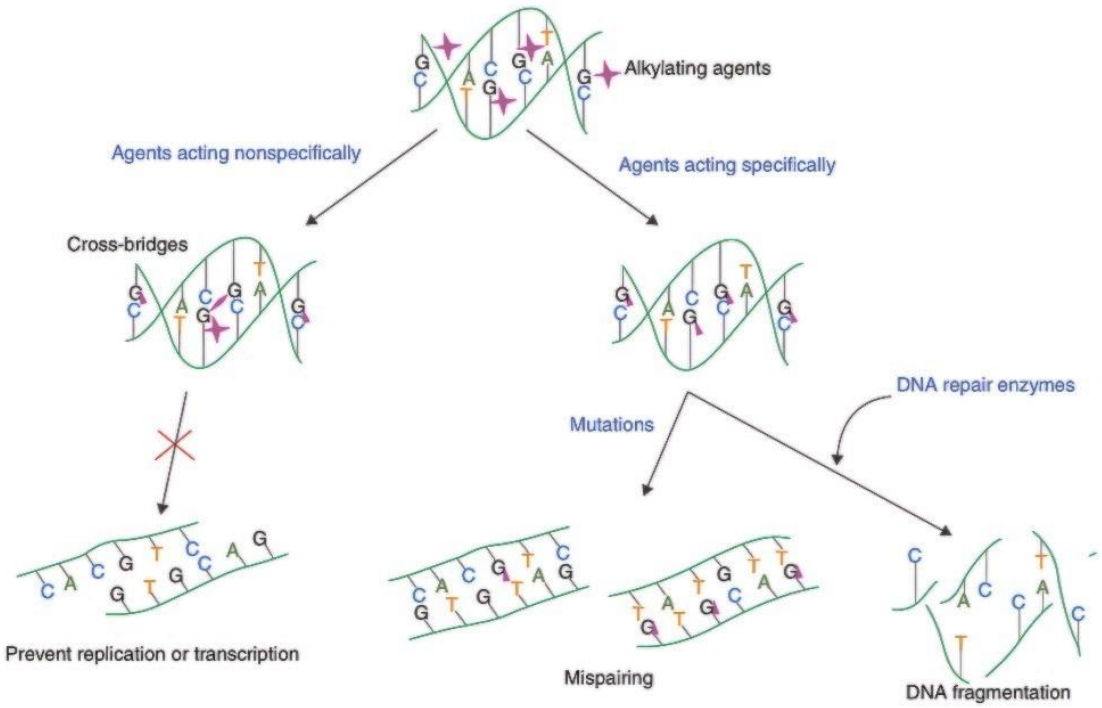

Figure 01: Action mechanism of alkylating agents **(Ralhan and Kaur, 2007).**

I.2.2. Antimetabolites

Antimetabolites are drugs that interfere with cellular metabolic processes due to their similarity to essential cellular molecules, particularly nucleosides and substrates of key enzymes **(Kaye, 1998).**

- **5-fluorouracil (5-FU)**

5-fluorouracil (5-FU) is a fluoropyrimidine belonging to the nucleoside analogue class. 5-FU is directly metabolized into 5-fluorouracil-3-phosphate (5-FUTP), which interferes with RNA synthesis by binding at the enzyme's active site, mimicking deoxyuridine mono-phosphate (dUMP), or it is converted into 5-fluorodeoxyuridine monophosphate, which is a specific inhibitor of thymidylate synthase (TS). This specific binding is followed by the incorporation of the folate cofactor 5, 10-methylenetetrahydrofolate, which locks the enzyme into an inhibited conformation similar to the transition state formed when converting dUMP into thymidine, consequently cellular levels of thymidine are diminished and the TS is unable to function normally **(Figure 02) (Albin, 2010; Kaye, 1998)**.

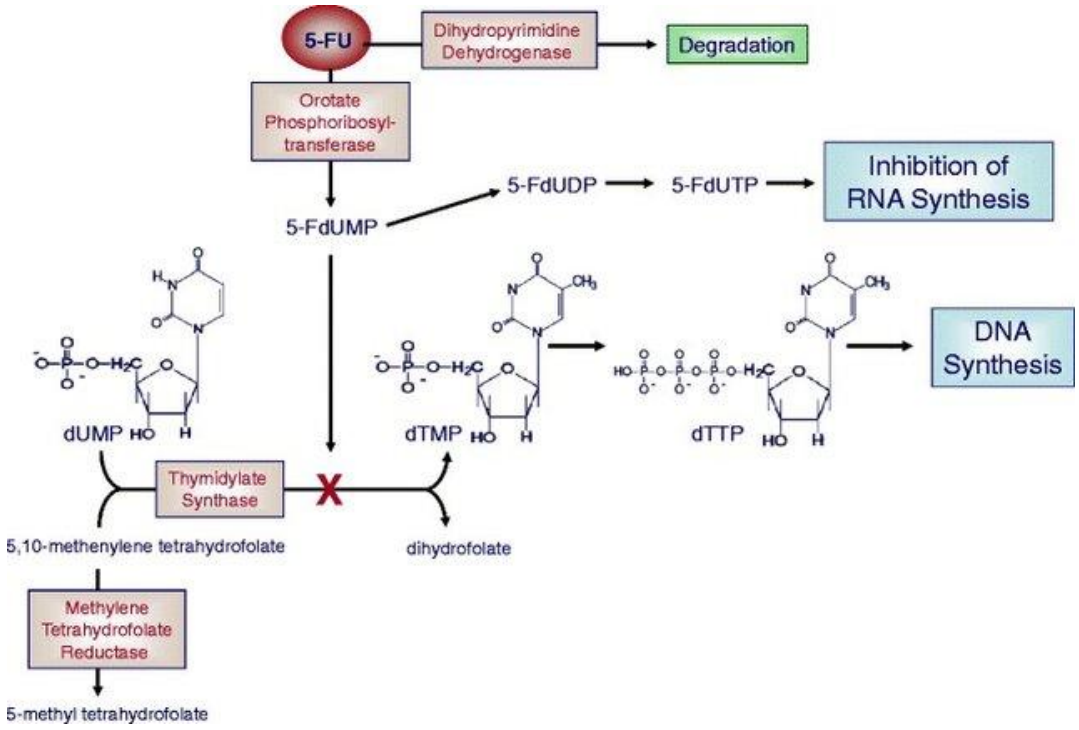

Figure 02: The 5-fluorouracil (5-FU) metabolism pathway **(Schwarzenbach, 2010).**

- **Capecitabine**

Capecitabine is a 5-FU prodrug; it is a cytidine analog that is administered orally and passes unchanged through the intestinal mucosa. It is activated in liver and tumor cells through

a series of enzymatic reactions and converted into 5-FU by thymidine phosphorylase, potentially in a tumor-selective manner **(Kaye, 1998).**

- **Gemcitabine**

Gemcitabine (Gemzar®) is a difluorinated deoxycytidine analog. After crossing the cell membrane, it is phosphorylated and activated by deoxycytidine kinase. The activated form inhibits DNA synthesis by incorporating at the end of the elongating DNA strand, only one more deoxynucleotide is added, and thereafter, the DNA polymerases are blocked. This masked termination action locks the drug into the DNA since the proofreading enzymes are incapable of eliminating gemcitabine from this position **(Figure 03) (Kaye, 1998; Plunkett et al., 1995).**

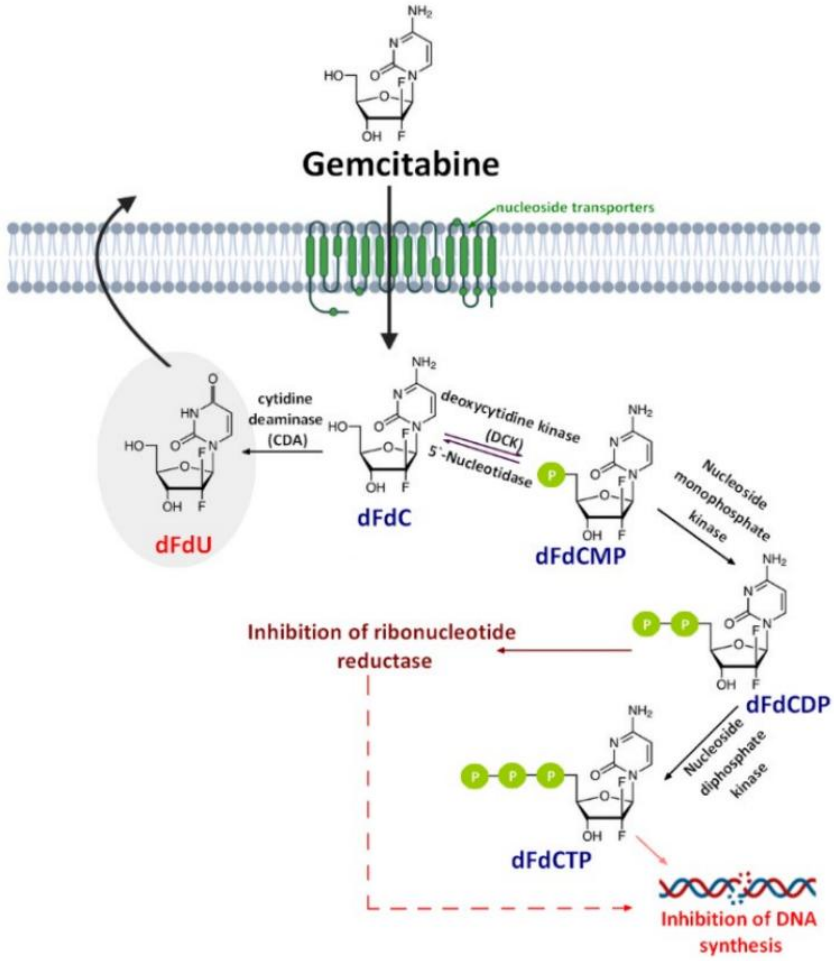

Figure 03: Action mechanism of Gemcitabine **(Hawryłkiewicz and Ptaszyńska, 2021).**

I.2.3. Anti-tumor antibiotics

Anthracyclines are either isolated from *Streptomyces peucetius,* such as doxorubicin (DOX) and daunorubicin (DNR), or semisynthetic analogs, including epirubicin (EPI) and idarubicin chloride.

DOX is one of the most effective neoplastic drugs administered either as monotherapy or in combination with other chemotherapy drugs. Antitumor effect of DOX results from its ability to intercalate into the DNA helix and/or bind to proteins involved in replication and transcription process, leading to cell death. Studies show that DOX enters the cell through simple diffusion and binds with high affinity to the proteasome. Afterwards, it binds to the 20S proteasomal subunit, forming a DOX-proteasome complex that translocates into the nucleus through ATP-dependent nuclear pores facilitated by a nuclear localization signal. Finally, the complex is dissociated, and DOX binds fto the DNA helix because of its higher affinity fto DNA than to the proteasome **(Carvalho et al., 2009).**

The damaged DNA is detected, leading to the activation of the *Ataxia telangiectasia* and Rad3-related kinase (ATR) and *Ataxia telangiectasia* mutated (ATM) pathways. Consequently, checkpoint 1 (CHK1) and checkpoint 2 (CHK2) are activated **(Figure 04-A).** These latest phosphorylate the phosphatases CDC25A and CDC25C, an inhibitory phosphorylation. As a result, CDK-cyclin are not dephosphorylated and prevent cell cycle progression **(Figure 04-B).** In addition, CHK2 phosphorylates the P53 transcription factor, regulating the P21 that binds active CDK-cyclin complexes resulting in cell arrest **(Figure 04-B) (Kciuk et al., 2023).** DOX is also classified as a topoisomerase II poison **(Carvalho et al., 2009).**

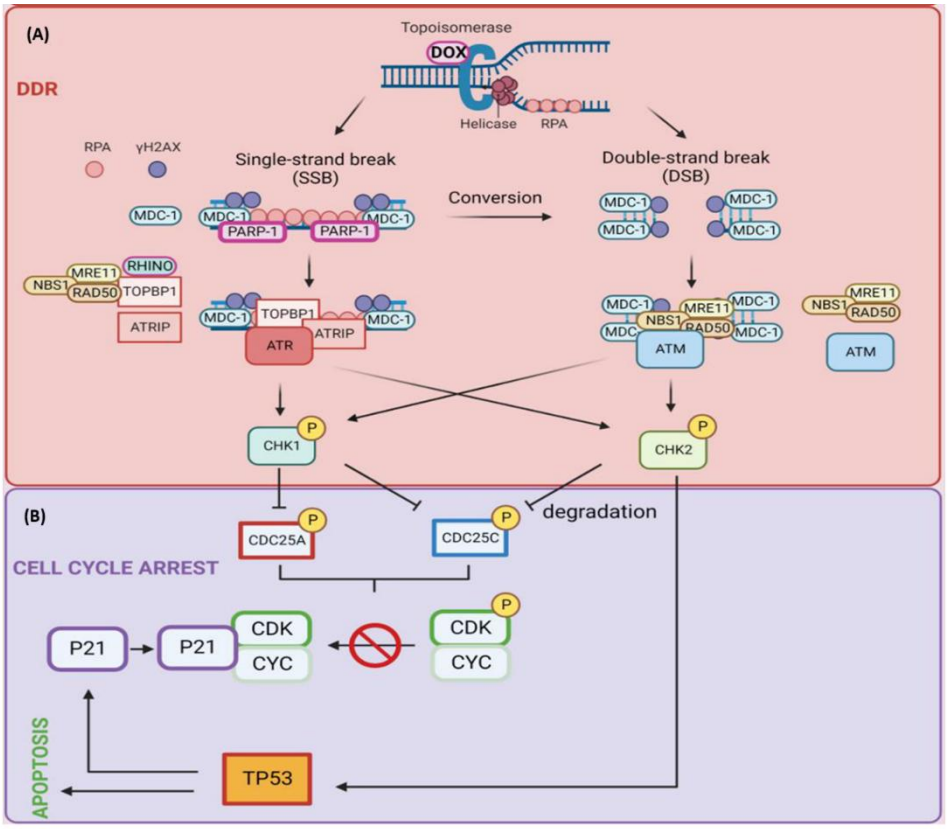

Figure 04: Action mechanism of Doxorubicin **(Kciuk et al., 2023).**

DOX triggers reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by binding to cardiolipin on the mitochondrial membrane **(Figure 05).** Thus, activating ATM-CHK2-P53 signaling independently of DNA damage. ROS activate P38, which activates P16, causing cell cycle arrest. Simultaneously, DOX increases P53 and decreases GATA4, altering gene transcription. This results in increased proapoptotic proteins (FASL, BAX, caspases 3/8, NOXA, PUMA) and decreased antiapoptotic factors (BCL-2, BCL-xL). Activation of the ceramide pathway and mitochondrial permeabilization via BAX and BAK release proapoptotic factors (APAF, SMAC/DIABLO, AIF) and procaspases, forming the apoptosome to activate caspases 3, 6, 7. DOX also activates extrinsic apoptosis by upregulating FASL, engaging death receptors (TNFR1, FAS, DR5), and activating CASP8 and effector caspases. **(Kciuk et al., 2023).**

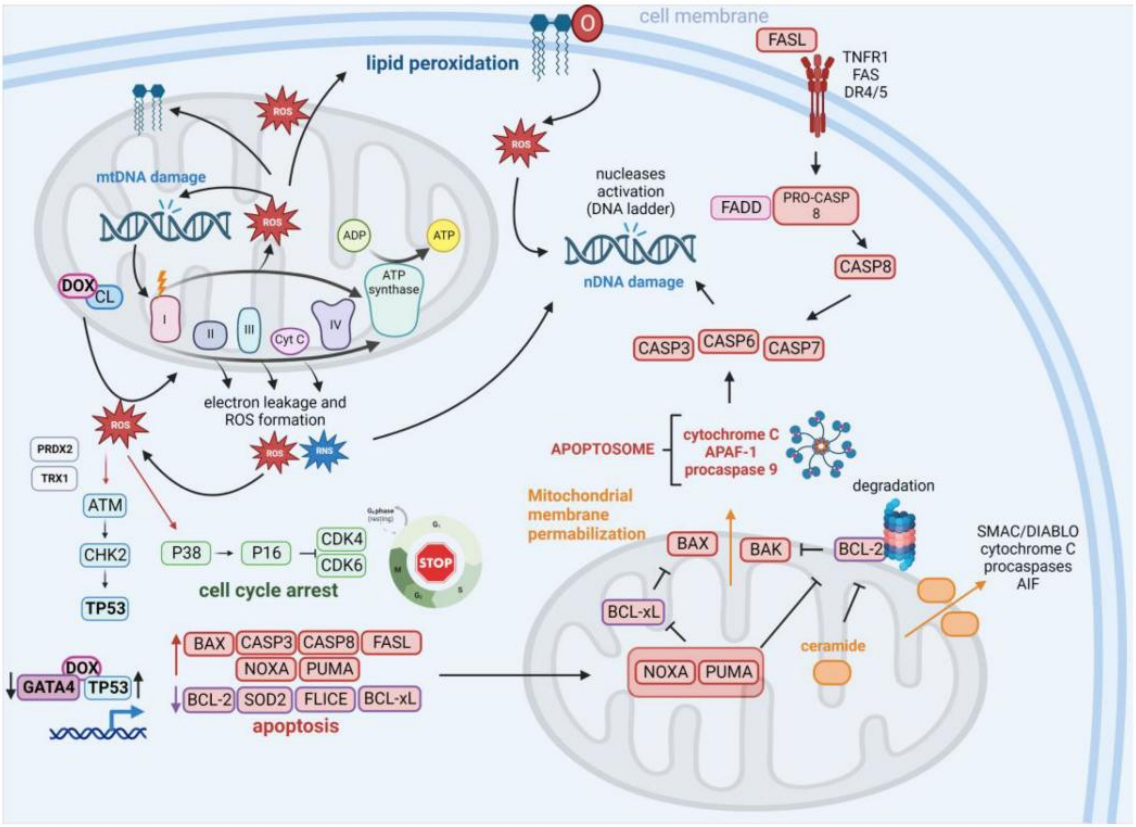

Figure 05: Contribution of DOX in ROS induction and apoptosis **(Kciuk et al., 2023).**

I.2.4. Topoisomerase inhibitors

Topoisomerases are nuclear enzymes that catalyze modifications of DNA topology, releasing the tension that occurs during transcription through the formation of transient single-stranded (topoisomerase I) or double-stranded (topoisomerase II) DNA breaks, rendering them essential enzymes for cell proliferation **(Skok et al., 2020).** Topoisomerase I (TOP1), inhibitors

such as irinotecan and topotecan, are derived from the natural alkaloid camptothecin. Their antitumor activity is due to their ability to poison the TOP1 cleavage complexes by being interfacial inhibitors **(Pommier and Thomas, 2023).**

Drugs targeting topoisomerase II (TOP2) are subdivided into two groups depending on their mechanism of action; the first group is TOP2 poisons including etoposide, doxorubicin, daunorubicin and mitoxantrone, they act through the stabilization of the covalent DNA-TOP2 complex acting as a cellular toxin by blocking replication and leading to DNA damage promoting cell death **(Skok et al., 2020)**.

The second group of drugs comprises TOP2 catalytic inhibitors. Their cytotoxicity involves the enzyme's inhibition without causing DNA damage either through competition for binding with ATP (novobiocin), preventing DNA cleavage (merbarone), or preventing the hydrolysis of ATP (dexrasoxane) **(Skok et al., 2020).**

I.2.5. Tubulin-binding drugs

Tubulin-binding drugs interfere with the heterodimerization of tubulin α and tubulin β, disrupting their dynamics. It leads to the daughter chromosomes' misalignment and the failure of attachment to the mitotic spindle. The cell fails to pass through the checkpoints that naturally ensure the proper progression of mitosis, resulting in the arrest of the cell cycle at the metaphase/anaphase transition and consequently triggering apoptosis **(Attard et al., 2006).**

Vinca alkaloids, including vinblastin, induce a curved tubulin assembly via their fixation to a binding site between the heterodimers **(figure 06) (Florian and Mitchison, 2016).**

Taxanes such as paclitaxel, a natural compound extracted from *Taxus brevifolia* and docetaxel, a semisynthetic compound derived from paclitaxel, are both known to be microtubule stabilizing drugs with the same antitumor activity. Their mechanism of action involves binding to a specific site in the microtubule's lumen causing over-stabilsation of the microtubles **(figure 06) (Florian and Mitchison, 2016).**

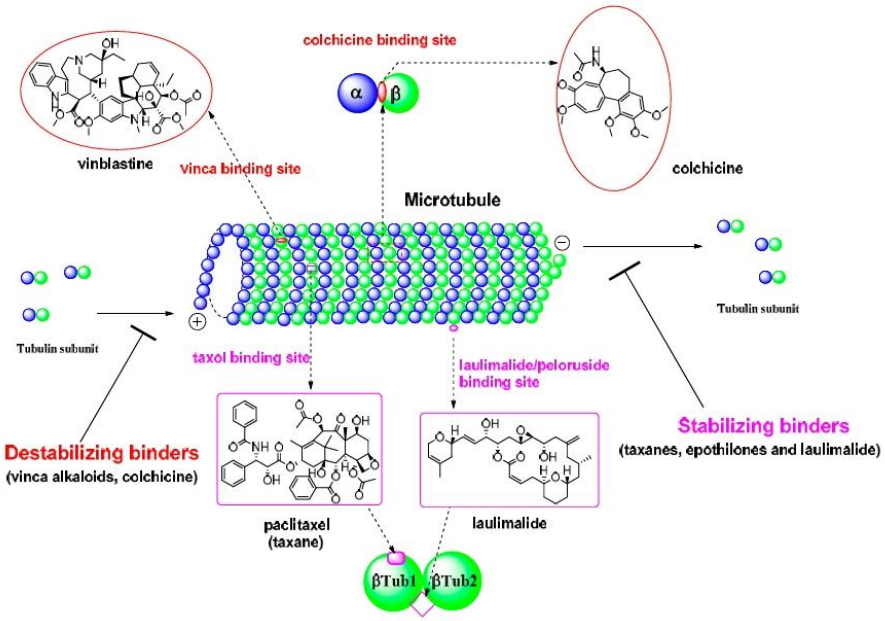

Figure 06: Tubulin-binding drugs binding sites **(Banerjee et al., 2016).**

I.3. Chemotherapy side effects

Anticancer chemotherapy is cytotoxic and inevitably causes damage to normal cells. It is responsible for gastrointestinal toxicity such as nausea, vomiting, diarrhea, and constipation. Oral toxicity is observed as well through necrotic mouth ulcers, mucositis, and other infections **(Lowenthal and Eaton, 1996).**

Anthracyclines may induce dose-dependent cardiotoxicity, while other drugs may cause acute arrhythmias (amsacrine), hemorrhagic myocardial necrosis (cyclophosphamide), angina (5FU), and bradycardia (paclitaxel). It also leads to liver damage and induces neurotoxicity, pulmonary toxicity, nephrotoxicity, and gonadal dysfunction. Alopecia is one of dermatological side effects as well as nail changes and skin pigmentation **(Lowenthal and Eaton, 1996).**

I.4. Hematotoxicity

I.4.1. Definition of hematotoxicity

Hematological toxicity refers to a reduction in bone marrow function and blood cell counts, which can result in infections, bleeding, or anemia **(Testart-Paillet et al., 2007).**

I.4.2. Hematotoxicity induced by chemotherapy

Anticancer chemotherapy causes hematotoxicity through two mechanisms: a direct effect on rapidly dividing cells and an indirect effect on the bone marrow microenvironment and hematopoietic growth factors. This toxicity affects erythrocytes, leukocytes, and thrombocytes, which leads to anemia, neutropenia, and thrombopenia **(Testart-Paillet et al., 2007).**

- **Anemia**

Anemia is defined as a reduction in baseline hemoglobin levels **(Table I)** and is the most common and persistent hematological abnormality in cancer patients. Chemotherapy-induced anemia results from several factors, including blood loss due to malignant invasion of normal tissues, bone marrow infiltration that disrupts erythropoiesis, and functional iron deficiency caused by inflammation **(Bryer and Henry, 2018).**

Table I : Classification of anemia by the National Cancer Institute **(Bryer and Henry, 2018)**.

Grade of anemia	Severity	HGB (g/dl)
0	Normal limits	12-16 for women/14-18 for men
1	Mild	10-12 for women/10-14 for men
2	Moderate	8-10 for both
3	Severe	6.5-8 for both
4	Life threatening	<6.5 for both

Chemotherapy can be immunosuppressive and inhibit erythropoiesis; some agents cause more severe degree of anemia than others. In a significant proportion of cancer patients with anemia the underlying cause is not clearly identifiable, this type of anemia is classified as "anemia of chronic illness". It is known to involve the activation of cytokines such as interferon-gamma, interleukin-1, and tumor necrosis factor (TNF), these cytokines can suppress the production of endogenous erythropoietin and inhibit the proliferation of erythroid precursor cells **(Abdel-Razeq and Hashem, 2020).**

Various treatment options are available, including blood transfusions, erythropoiesis-stimulating agents, and intravenous iron therapy **(Abdel-Razeq and Hashem, 2020).**

- **Neutropenia**

Neutropenia is defined as a laboratory analysis indicating a decreased number of neutrophils in blood sample **(Table II) (Fontanella et al., 2014).**

Table II : Neutropenia grades **(Crawford et al., 2003)**.

Grade of neutropenia	Value ($\times 10^9$ /L)
0	1.5 - 8.0
1	≥ 1.5 to <2.0
2	≥ 1.0 to <1.5
3	≥ 0.5 to <1.0
4	< 0.5

Neutropenia is a common side effect of chemotherapy that occurs when myelosuppressive drugs lower the absolute neutrophil count. The duration of neutropenia usually ranges from 7 to 10 days. However, this can vary depending on the type and intensity of chemotherapy, as well as patient-specific factors such as bone marrow reserve, cancer type, existing comorbidities, and age **(Caggiano et al., 2005).** Drug-induced neutropenia is primarily caused by the direct suppression of bone marrow precursor cells **(Fontanella et al., 2014).**

Granulocyte colony-stimulating factors (G-CSF) based therapies function by promoting the release of mature neutrophils into the bloodstream, accelerating the development of neutrophil precursors in the bone marrow, and preventing infections, reducing antibiotic use and hospital admissions **(Blayney, 2022).**

- **Thrombopenia**

Thrombopenia may be caused by the disease itself or one of its symptoms. However, chemotherapy that suppresses bone marrow functions is the most common cause, and this can lead to fatal bleeding **(Gao et al., 2023).** The incidence of chemotherapy induced thrombopenia varies significantly depending on the treatment regimen and patient demographics, factors such as age, type of therapy and cancer type, each influence its occurrence differently **(Table III) (Gao et al., 2023).** In cases of sever chemotherapy induced thrombopenia, the primary objective is to prevent bleeding. Vitamin-K may be given to correct blood clotting in patients on warfarin or those lacking vitamin-K dependent coagulation factors **(Gao et al., 2023).** Platelet transfusion often remains the only readily available immediate treatment option **(Gao et al., 2023).**

Table III: Thrombopenia grades (According to the National Cancer Institute Common Toxicity Criteria, version 2.0)

Grade	Value ($\times 10^9$ /L)
0	150 – 450
1	< 75.0
2	≥ 50.0 to < 75.0
3	≥ 10.0 to < 50.0
4	< 10.0

A recapitulation of selected studies on hematotoxicity is presented in Table IV.

Table IV: Summary of selected studies on hematotoxicity.

Object of study	Hematological parameters	Results
The study aimed to evaluate whether hematologic risk differed between patients treated with carboplatin and those who were not exposed to it **(Cheng et al., 2017).**	↓ Erythrocytes ↓ Neutrophils ↓ Platelets	- Carboplatin was more often reported for anemia, neutropenia and thrombopenia **(Cheng et al., 2017).** - A highly significant enrichment indicates that carboplatin significantly impacts blood cell development by disrupting specific key genes **(Cheng et al., 2017).**
This study investigates the early transformation of erythrocytes following cytotoxic injury induced by paclitaxel, carboplatin, doxorubicin, and cyclophosphamide *in vitro* Using laser diffraction, flow cytometry, and confocal	Erythrocytes	- Paclitaxel, which targets cytoskeletal proteins caused the most severe erythrocyte abnormalities, such as impaired volume regulation, osmotic resistance and stomatocytosis especially when combined with

microscopy **(Skverchinskaya et al., 2023).**		carboplatin **(Skverchinskaya et al., 2023).** - Microfluidic simulations revealed slow moving damaged cells and more frequent occlusions (**Skverchinskaya et al., 2023**). - In contrast, DNA targeting drugs like carboplatin, cyclophosphamide and doxorubicin showed lower short-term cytotoxicity to red blood cells **(Skverchinskaya et al., 2023).** -However, drug combinations produced additive toxic effects **(Skverchinskaya et al., 2023).**
This study aimed to evaluate the effects of various chemotherapy drugs such as cisplatin, 5-FU, and sunitinib on red blood cell deformability, aggregation, and suspension fluidity **(Muravyov et al., 2016).**	Erythrocytes	- Cisplatin and epoetin alpha improved RBC microrheology likely via activation of tyrosine protein kinase **(Muravyov et al., 2016).** - 5-FU increased RBC aggregation which was

		reversed by calcium chelation and pentoxifylline. - Sunitinib significantly raised RBC aggregation but had a little effect on deformability; its combination with cisplatin neutralized the pro-aggregative effect **(Muravyov et al., 2016).**

Chapter II

Oxidative stress

II. Oxidative stress

II.1. Definition

Oxidative stress (OS) is defined as an excessive production of reactive oxygen species (ROS) that cannot be completely neutralized by antioxidants, as well as a disruption of the cellular redox balance **(Pisoschi and Pop, 2015).**

II.2. Reactive oxygen species

Reactive oxygen species (ROS) **(Table V)** are highly reactive molecules produced during oxygen metabolism; they can exist in either free radical or non-radical forms. Free radicals contain at least one unpaired valence electron in their outer shell, which makes them extremely reactive and short-lived **(Ahmad et al., 2017).**

At low concentration ROS act as a signaling molecules involved in regulating cell proliferation, apoptosis, and gene expression by activating transcription factors **(Pisoschi and Pop, 2015).**

Table V: Example of ROS **(Ahmad et al., 2017)**.

Free radicals	Non radicals
Hydroxyl radical (•OH)	Hydrogen peroxide (H_2O_2)
Superoxide anion ($•O_2$-)	Singlet oxygen ($^1O_2$)
Lipid peroxyl (•LOO-)	Ozone (O_3)
Thiyl (•RS)	Lipid peroxide (LOOH)
	Peroxynitrite ($ONOO^-$)

II.3. Sources of reactive oxygen species

ROS are generated through various sources, which are divided into two categories: endogenous and exogenous **(Pisoschi and Pop, 2015).**

II.3.1. Endogenous sources of production of ROS

Mitochondria are a primary source of ROS, responsible for generating nearly 90% of the total ROS produced within the cell in the respiratory chain **(Figure 07) (Kausar et al., 2018).** In addition enzymatic activity such as peroxysomes, and the exposure to microbial infections involving phagocytosis can generate ROS within the cells **(Pisoschi and Pop, 2015).**

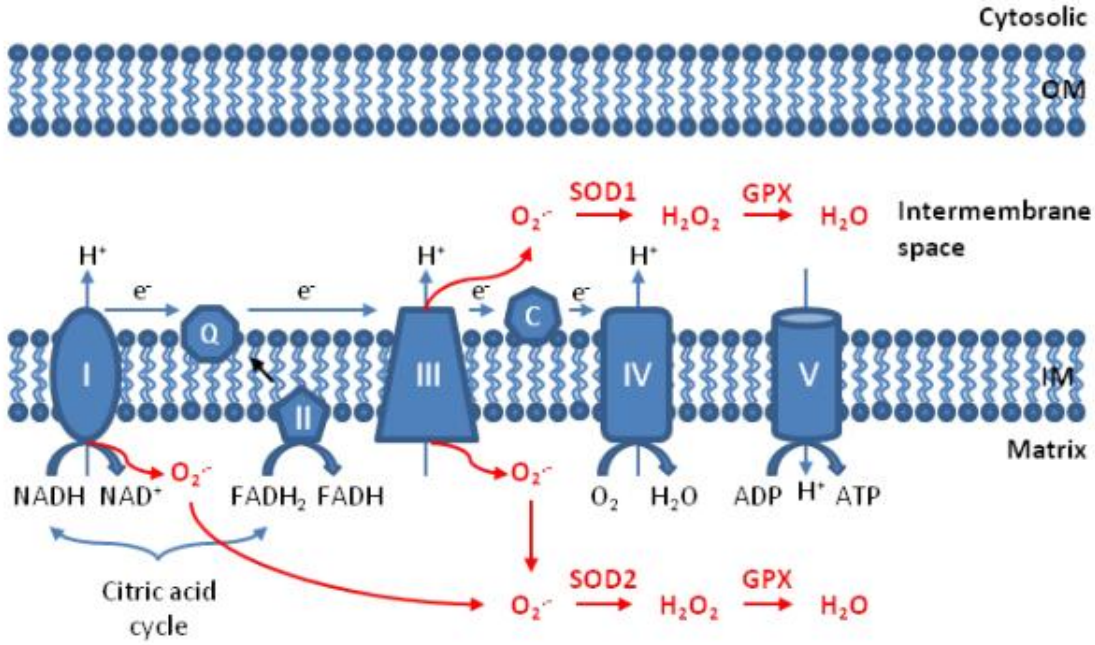

Figure 07: Major sites for the production of reactive oxygen species in mitochondria **(Li et al., 2013).**

II.3.2. Exogenous sources of production of ROS

Environmental factors are primary contributors to oxidative stress, resulting in increased production of ROS. Among these factors: cigarette smoke, alcohol, ionizing and UV radiation, pesticides, and ozone. **(Pisoschi and Pop, 2015).**

II.4. Oxidative stress damage

Oxidative stress primarily induces cell damage through three main mechanisms: membrane lipid peroxidation, protein oxidation, and DNA damage **(Zhang et al., 2018).**

II.4.1. Lipid peroxidation

The chain reaction of lipid peroxidation is a process that affects cell membranes and other lipid-containing structures. Hydroperoxides are critical intermediates in this reaction, capable of disrupting membrane integrity and posing a threat to the cell (Zhang et al., 2018). Aldehydes such as malonaldehydes (MDA) and 4-hydroxynonenal 1/4-hydroxy-2-nonenal (HNE) (figure 09) are secondary products recognized as biomarkers of oxidative stress. Their uncharged molecular structure enables them to readily cross cellular membranes and enter the cytosol. This property allows them to exert widespread and profound damaging effects both within and beyond the cell, often surpassing the impact of ROS **(Zhang et al., 2018).**

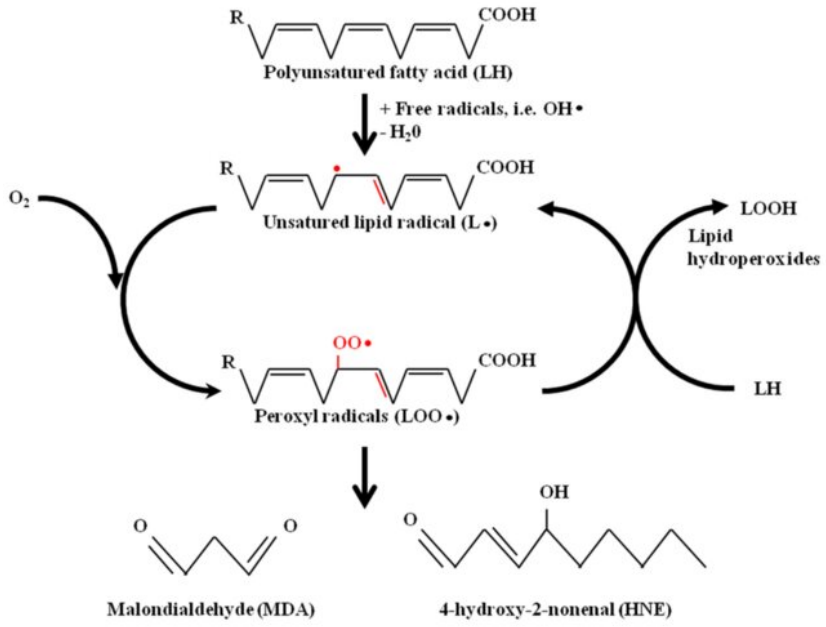

Figure 08: Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) formation **(Barrera et al., 2018).**

II.4.2. Protein oxidation

When proteins are exposed to radical attack in the presence of ROS, a variety of structural and chemical modifications can occur. These include side chain-oxidation, backbone fragmentation, cross-linking, unfolding, changes in hydrophobicity and conformation, altered sensitivity to proteolytic enzymes, and the formation of new groups such as reactive carbonyls, hydroperoxides, and 3,4-dihydroxyphenilalanine. Ultimately, these alterations can lead to a loss of the protein's structural integrity or enzymatic functions, resulting in biological disruptions **(Headlam and Davies, 2004).**

II.4.3. DNA damage

A major effect of ROS is its ability to directly damage DNA. Endogenous DNA damage may involve attacks on DNA bases or deoxyribose residues, resulting in base modifications or strand breaks. Also, DNA lesions are genotoxic and often lead to mutations commonly found in altered protooncogenes. 8-oxo-deoxyguanosine is one of the most extensively studied oxidative DNA lesions, playing a key role in the induction of spontaneous mutations that lead to misincorporation by DNA polymerases, most commonly resulting in "Guanine" to "Thymine" transversions, which are frequently observed in mutated oncogenes and tumor suppressor genes **(Kang, 2002).**

II.4.4. Oxidative stress and chemotherapy

Chemotherapy-induced oxidative stress is now recognized as a major contributor to organ injury, significantly impacting organ function and overall treatment tolerability **(Yarana and St. Clair, 2017).** Damage to healthy tissues, particularly chemotherapy-induced oxidative stress which causes cardiomyopathy, is an unintended consequence that can have severe and harmful effects on health **(Yarana and St. Clair, 2017).**

In the USA, about half of the cancer treatment drugs approved by the Food and Drug Administration (FDA) are associated with the production of ROS, with doxorubicin (DOX) being one of these **(Yarana and St. Clair, 2017).**

- **Oxidative stress and hematotoxicity**

Reactive oxygen species play a dual role in tumorigenesis, especially in hematologic malignancies. On one hand, ROS can trigger cell death processes such as apoptosis, which can be leveraged for cancer treatment. On the other hand, they can also promote carcinogenesis by protecting cells from apoptosis, enhancing cell survival, and inducing processes such as proliferation, migration, metastasis, and drug resistance. It has been reported that oxidative stress is involved in the development of various hematologic malignancies, including acute myeloid leukemia and chronic myeloid leukemia. Different therapeutic approaches, such as chemotherapy, are known to induce ROS or other free radicals in patients receiving cancer treatment. Evidence suggests that leukemia stem cells are more reliant on oxidative respiration and are more sensitive to oxidative stress compared to normal hematopoietic stem cells. While oxidative stress has been linked to the etiology and progression of leukemia, many chemotherapeutic agents achieve their biological effects by inducing oxidative stress in affected cells **(Zhang et al., 2018).**

Current leukemia treatment mainly involves high-dose cytotoxic chemotherapy; these chemotherapeutic regimens often lead to increased levels of ROS, which can result in drug intolerance or resistance. The underlying mechanisms are likely driven by ROS-dependent pathways. Chemotherapy interferes with mitotic and metabolic functions in cells, leading to disrupted signal transduction and damage to subcellular organelles, which in turn contributes to excessive ROS production **(Zhang et al., 2018).**

Chapter III

Materials and methods

III. Materials and methods

III.1. Materials

III.1.1. Equipment

Centrifuge (Eppendorf®); vortex (VELP SCIENTIFICA®); balance (QIAS®); magnetic agitator (Raypa®); spectrophotometer UV-Vis (JENWAY Genova®); microscope (ZEISS West Germany®); water bath (Raypa®); cell blood count analyzer (Swelab Alpha®).

III.1.2. Chemical products

All chemical products below are from PROCHIMA-SIGMA :

-Sodium chloride (NaCl).

-Trichloroacetic acid (TCA).

-Sodium hydroxyde (NaOH).

-Thiobarbituric acid (TBA).

-Ethylene diamine teraacetic acid (EDTA).

-2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

-Potassium persulfate ($K_2S_2O_8$).

-Giemsa.

-Ethanol (96%).

III.1.3. Sampling

Samples were collected in EDTA tubes before and after chemotherapy from twenty-eight patients, but only thirteen patients were kept **(Table VI)** due to treatment side effects and clotting issues. The patients are from different ages (52.41±15.21 years old), sexes, cancer types, and chemotherapy regimens at the medical oncology unit of the CHU of Bejaia.

Table VI: Patients' clinical data.

Patient	Sexe	Age	Cancer type	Regimen	Duration
P1	female	45	breast	DOX/CYP	1h
P2	female	53	breast	DOX/CYP	1h
P3	female	21	breast	TXT	1h
P4	male	76	lung	CBDCA	1h
P5	male	73	bladder	dFdC	30min
P6	male	29	colon	dFdC	30min
P7	male	71	colon	5FU	1h
P8	female	61	ovary	dFdC	30min
P9	female	42	breast	TXT	1h
P10	male	57	rectum	5FU	1h
P11	male	35	rectum	folinic acid/5FU/L-OHP	2h
P12	male	53	synovial	TXT	1h
P13	male	34	colon	folinic acid/5FU/L-OHP	2h

All patients as well as oncology unit head doctor signed a concent put in annexe.

III.2. Methods

III.2.1. Blood cell count

Blood cell count was performed both before and after chemotherapy in a blood cell analyzer in Bejaia CHU's central laboratory **(Figure 09).**

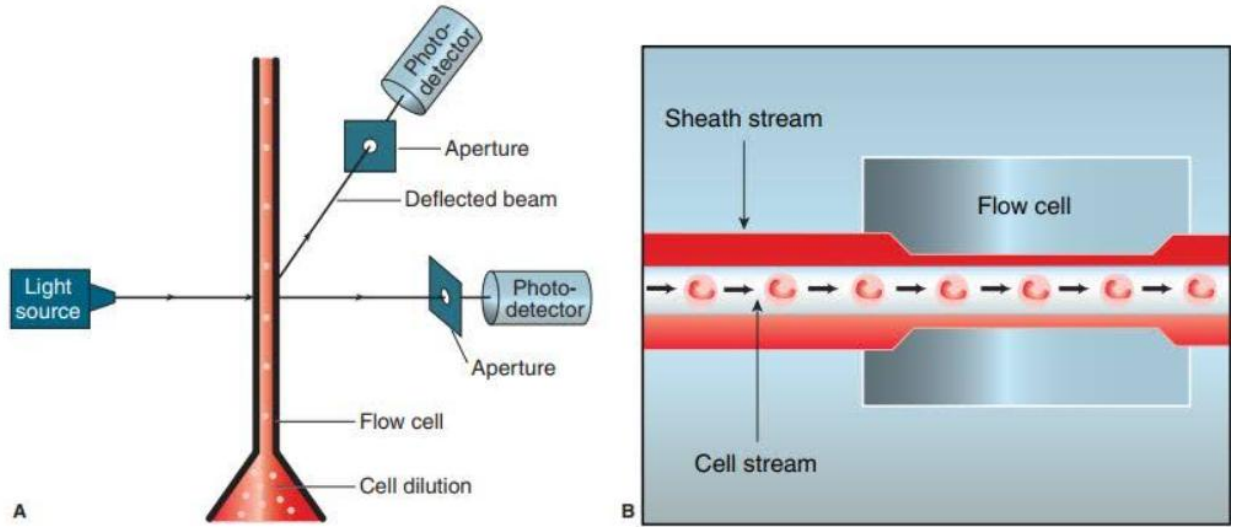

Figure 09: Blood cell analizer principle **(clinicalsci, 2020).**

III.2.2. Blood smear

5 µl of blood has been spread on microscope slides, air-dried, then fixed with ethanol (96%), air-dried again, and stained using Giemsa (1/2, v/v) for 20 minutes, rinsed thoroughly with water. The blood smear slides were observed under an optical microscope at a magnification of G×10×40 **(Figure 10).**

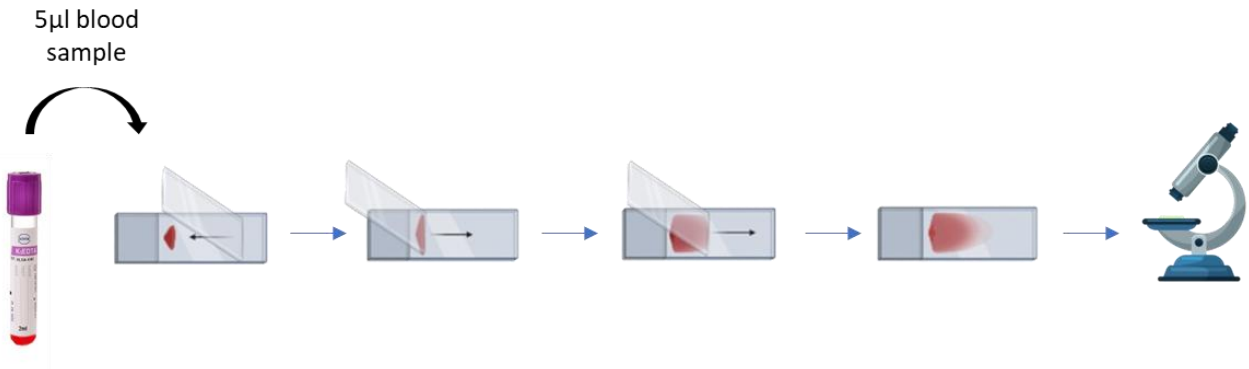

Figure 10: Schematic of blood smear method.

III.2.3. Blood separation

Blood samples were separated using an Eppendorf centrifuge at 3000 rpm for 10 minutes at 4°C. Plasma and pellets were transferred separately to Eppendorf tubes and stored at -18°C **(Figure 11).**

Figure 11: Schematic of blood samples separation.

III.2.4. Oxydative stress assay

- **Malondialdehyde (MDA) measurement**

Blood samples were diluted as previously described with a ratio of 1:5 for plasma and 1:10 for pellets. The diluted sample was incubated with trichloroacetic acid (TCA) for 2 hours in ice bath. After incubation, a centrifugation was done at 3000 rpm for 10 minutes at 4°C. Thiobarbituric acid (TBA) and EDTA were added to the supernatant, which was then incubated at 95°C for 15 minutes (Figure 14). This was followed immediately by cooling the mixture in an ice bath. The absorbance was measured at 535nm using a JENWAY Genova ®spectrophotometer **(Figure 12) (Mameri et al., 2021).**

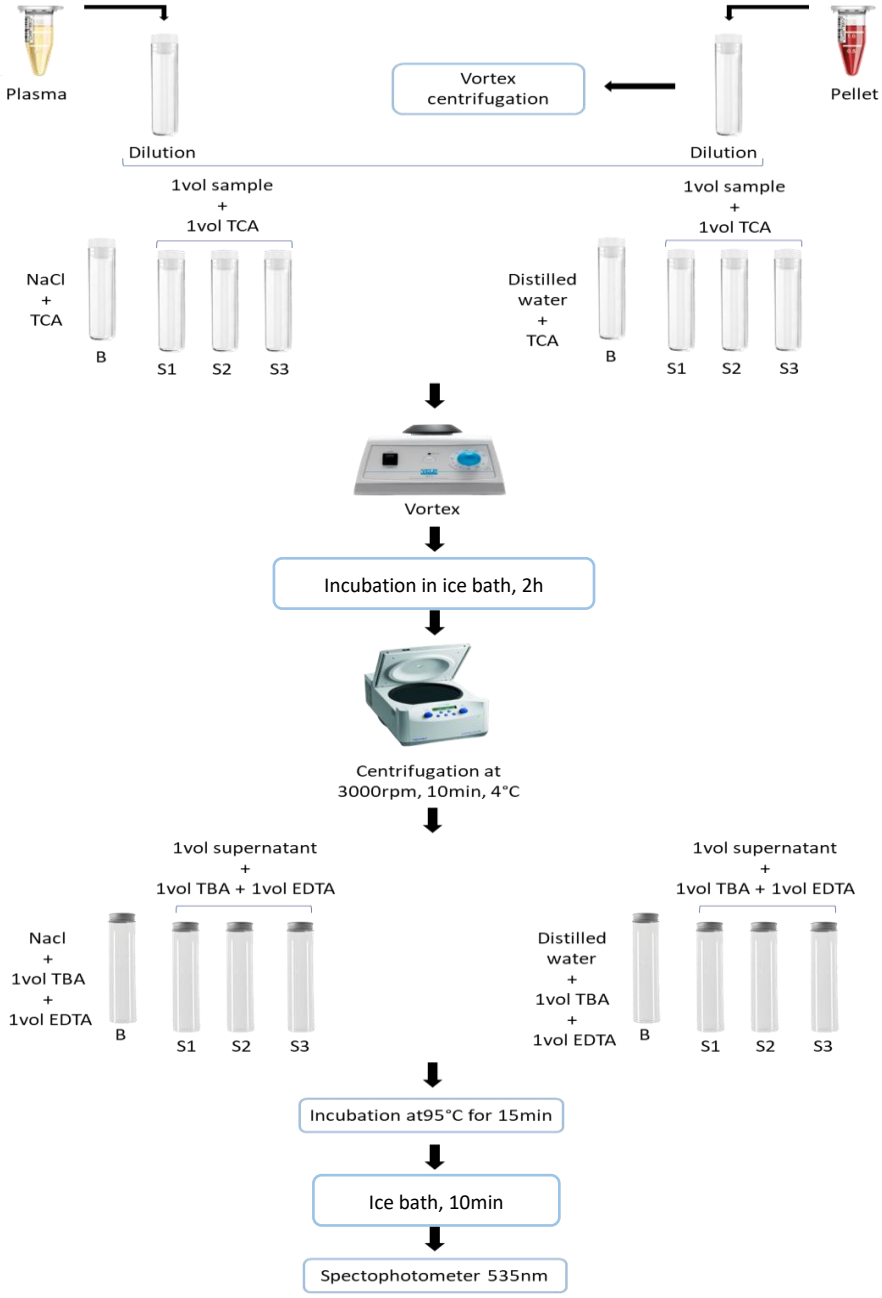

Figure 12: TBARs protocol.

- **Measurement of hemoglobin and methemoglobin**

Plasma and pellets were diluted using NaCl and distilled water, respectively, with a ratio of 1:5 for plasma and 1:2000 for pellets. The absorbance of the diluted samples was measured using a JENWAY Genova spectrophotometer at two wavelengths: 412 nm for hemoglobin measurement and 540 nm for methemoglobin measurement **(Figure 13).**

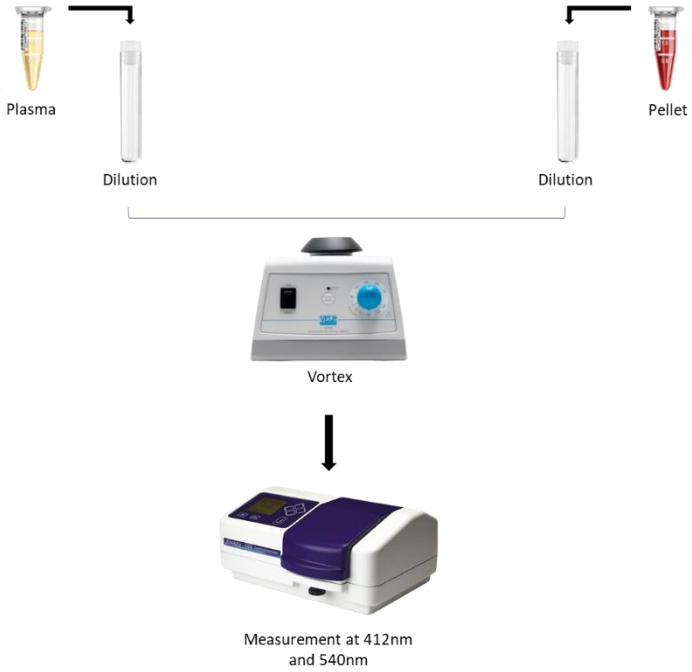

Figure 13: Schematic of hemoglobin and methemoglobin measurement.

- **Total antioxydant capacity**

The ABTS test was performed on both diluted plasma and pellets with a ratio of 1:5 and 1:10, respectively. ABTS solution was previously prepared using a volume of ABTS (7 mM) and potassium persulfate (2.45 mM). The mixture was incubated for 12 to 16 hours at room temperature in the dark **(Re et al., 1999).** The ABTS solution was diluted until reaching an absorbance of 0.700 ± 0.02 at 734nm. 1ml of the diluted ABTS solution was incubated with 10µl of diluted samples for 6 minutes in the dark. The absorbance was measured at 734 nm using a JENWAY Genova® spectrophotometer **(Figure 14).**

The percentage of inhibition of ABTS was calculated using the following equation:

$$\%\ \textbf{inhibition ABTS°} = \frac{\text{ABTS Abs} - \text{Sample Abs}}{\text{ABTS Abs}} \times 100$$

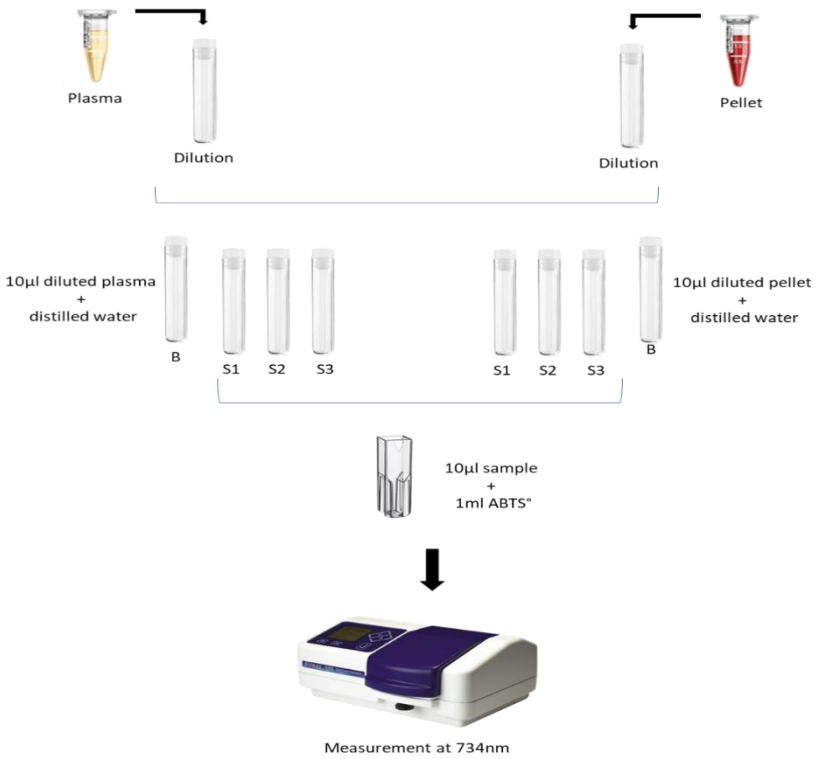

Figure 14: ABTS° test protocol.

III.3. Statistical analysis

All experiments were performed in triplicate. Results are expressed as means ± SEM. Differences among groups were assessed using one-way analysis of variance (ANOVA), followed by an unpaired Student's t-test. Differences were considered statistically significant when $P < 0.05$. Statistical analysis was performed using STATVIEW software (SAS Institute Inc., Version 5).

Chapter IV

Results and discussion

IV. Results and discussion

IV.1. Results

IV.1.1. Blood cell count

Figure 15 illustrates the changes in white blood cells (WBC) before and after chemotherapy. The results indicated that all treatments, including 5FU, CBDCA, dFdC, and TXT, decreased the WBC count ($4.40\pm0.00\times10^9$/L; $5.70\pm0.00\times10^9$/L; $3.70\pm1.212\times10^9$/L, and $4.70\pm3.067\times10^9$/L, respectively) after receiving the treatment compared to before ($5.10\pm1.424\times10^9$/L; $5.80\pm0.00\times10^9$/L; $3.90\pm0.999\times10^9$/L, and $5.05\pm2.574\times10^9$/L, respectively). In contrast, treatment with the combination of DOX/CYP increased WBC count singnificantly (p=0.0108), along with the polytherapy composed of folinic acid/5FU/L-OHP ($11.95\pm1.041\times10^9$/L and $4.20\pm0.548\times10^9$/L, respectively) compared to before receiving the chemotherapy ($7.85\pm0.274\times10^9$/L and $3.55\pm1.041\times10^9$/L, respectively).

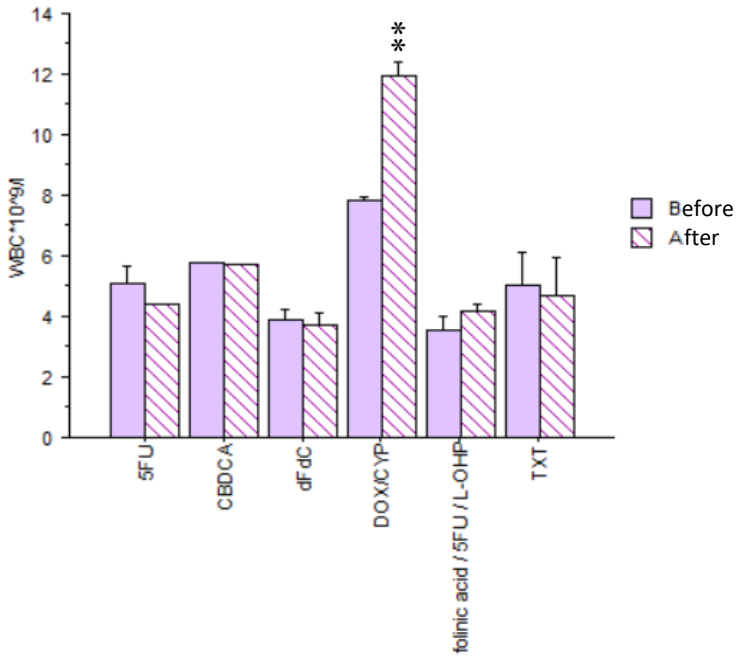

Figure 15: White blood cell count before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

The variation in lymphocyte count before and after chemotherapy is presented in **Figure 16**. The results showed that the administration of 5FU, dFdC, the combination of DOX/CYP, and the polytherapy folinic acid/5FU/L-OHP caused a decrease in lymphocyte count ($0.45\pm0.164\times10^9$/L; $0.733\pm0.050\times10^9$/L; $1.70\pm0.438\times10^9$/L, and $0.55\pm0.167\times10^9$/L, respectively) when compared to before receiving the treatment ($0.85\pm0.164\times10^9$/L; $1.367\pm0.200\times10^9$/L; $1.95\pm0.164\times10^9$/L, and $0.90\pm0.110\times10^9$/L, respectively), significantly

dFdC (p=0.0007) and DOX/CYP (p=0.0505). In contrast, TXT resulted in an increase in lymphocyte count ($0.95\pm0.383\times10^9$/L) compared to before treatment ($0.90\pm0.657\times10^9$/L). Whereas CBDCA administration showed no difference before and after chemotherapy ($0.70\pm0.00\times10^9$/L).

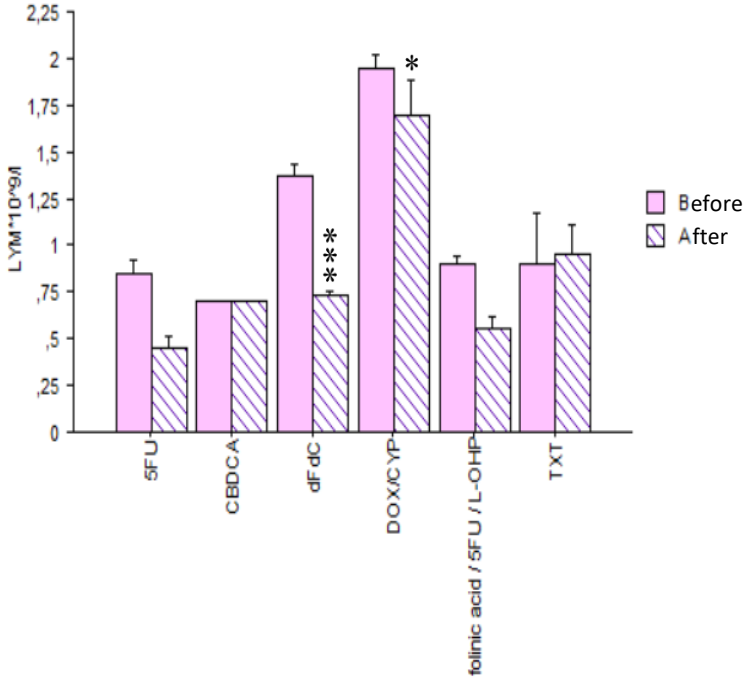

Figure 16: Lymphocyte count before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

The results illustrated in **Figure 17** demonstrate the variation in RBC count before and after chemotherapy. The findings indicated that 5FU significantly decreased RBC count (p=0,0004), along with dFdC, and TXT ($3.22\pm0.756\times10^{12}$/L, $3.443\pm0.367\times10^{12}$/L, and $3.115\pm0.126\times10^{12}$/L, respectively) compared to before the treatment ($4\pm0.099\times10^{12}$/L, $3.623\pm0.291\times10^{12}$/L, and $3.19\pm0.329\times10^{12}$/L, respectively). In contrast, treatment with CBDCA, the combination of DOX/CYP, and folinic acid/5FU/L-OHP showed an elevation in RBC count ($4.11\pm0.00\times10^{12}$/L; $4.224\pm0.383\times10^{12}$/L and $4.46\pm0.219\times10^{12}$/L, respectively) compared to before treatment ($3.84\pm0.00\times10^{12}$/L; $4.17\pm0.416\times10^{12}$/L, and $4.425\pm0.433\times10^{12}$/L, respectively).

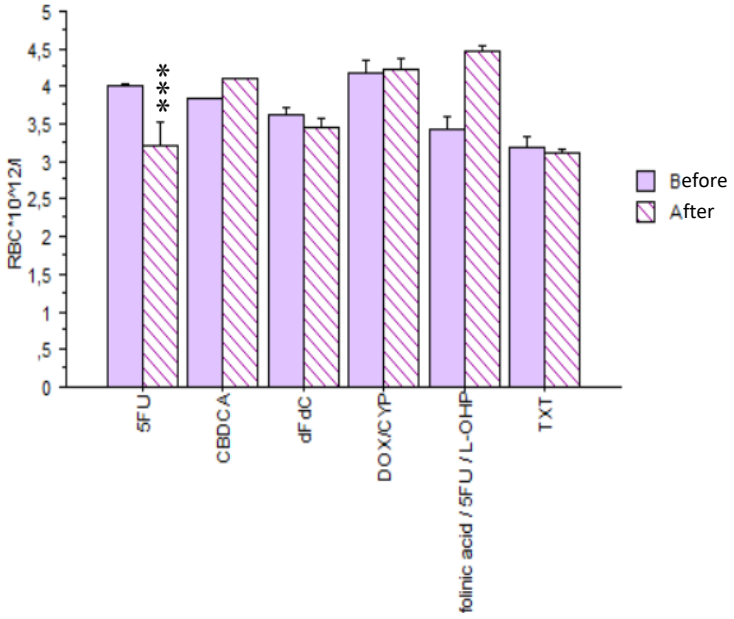

Figure 17: Red blood cell count before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

The platelet count before and after chemotherapy is illustrated in **Figure 18**. A significant decrease was registered after DOX/CYP administration ($p<0.0001$) ($222\pm102.972\times10^9$/L) versus before treatment ($317\pm9.859\times10^9$/L), while a slight decrease was observed after the administration of 5FU and TXT ($114.50\pm77.229\times10^9$/L and $212\pm73.943\times10^9$/L, respectively) compared to before treatment ($125.50\pm44.366\times10^9$/L and $218.67\pm74.490\times10^9$/L, respectively). However, treatment with dFdC and significantly folinic acid/5FU/L-OHP polytherapy ($p=0.02$) increased the platelet number ($129.333\pm55.953\times10^9$/L and $150\pm28.482\times10^9$/L, respectively) compared to the results obtained before the treatment ($112.667\pm47.547\times10^9$/L and $132.50\pm9.311\times10^9$/L, respectively). In contrast, no variation was observed after CBDCA chemotherapy ($157\pm0.00\times10^9$/L).

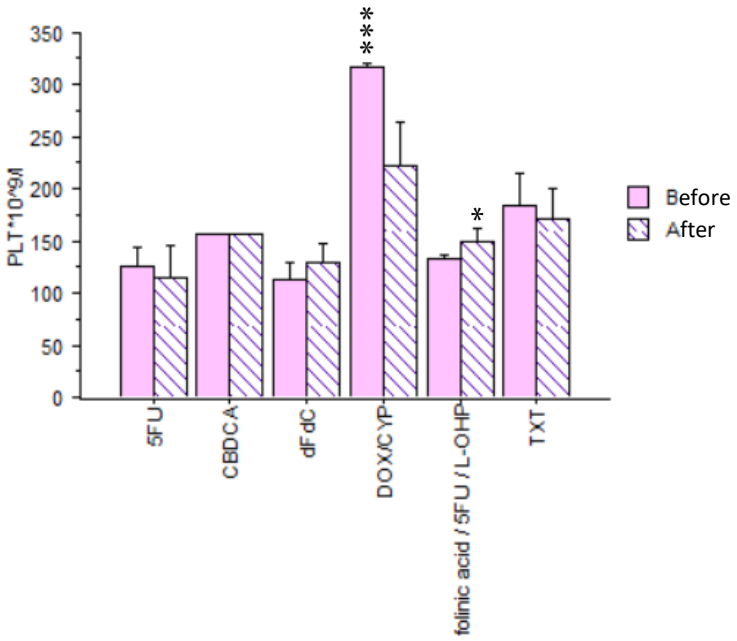

Figure 18: Platelet count before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

IV.1.2. Blood smear

Figure 19 illustrates peripheral blood smears realized before and after chemotherapy. The results showed that blood cells before chemotherapy were predominantly normocytic, while anisopoikilocytosis (a change in size and shape) was observed following chemotherapy. Rouleaux formation, indicating RBC aggregation, as well as stomatocytes, were noted after administering all chemotherapeutic agents. Additionally, dacrocytes (teardrop cells) appeared after the administration of 5FU, the combination of DOX/CYP, and TXT. Furthermore, elliptocytes were observed following treatment with CBDCA, as well as in regimens combining DOX/CYP and polytherapy with folinic acid/5FU/L-OHP. Moreover, microcytes were visible after receiving the combination of folinic acid/5FU/L-OHP and TXT. Schistocytes were also observed after treatment with CBDCA and dFdC.

Lysed lymphocytes were observed on peripheral blood smears of patients treated with dFdC.

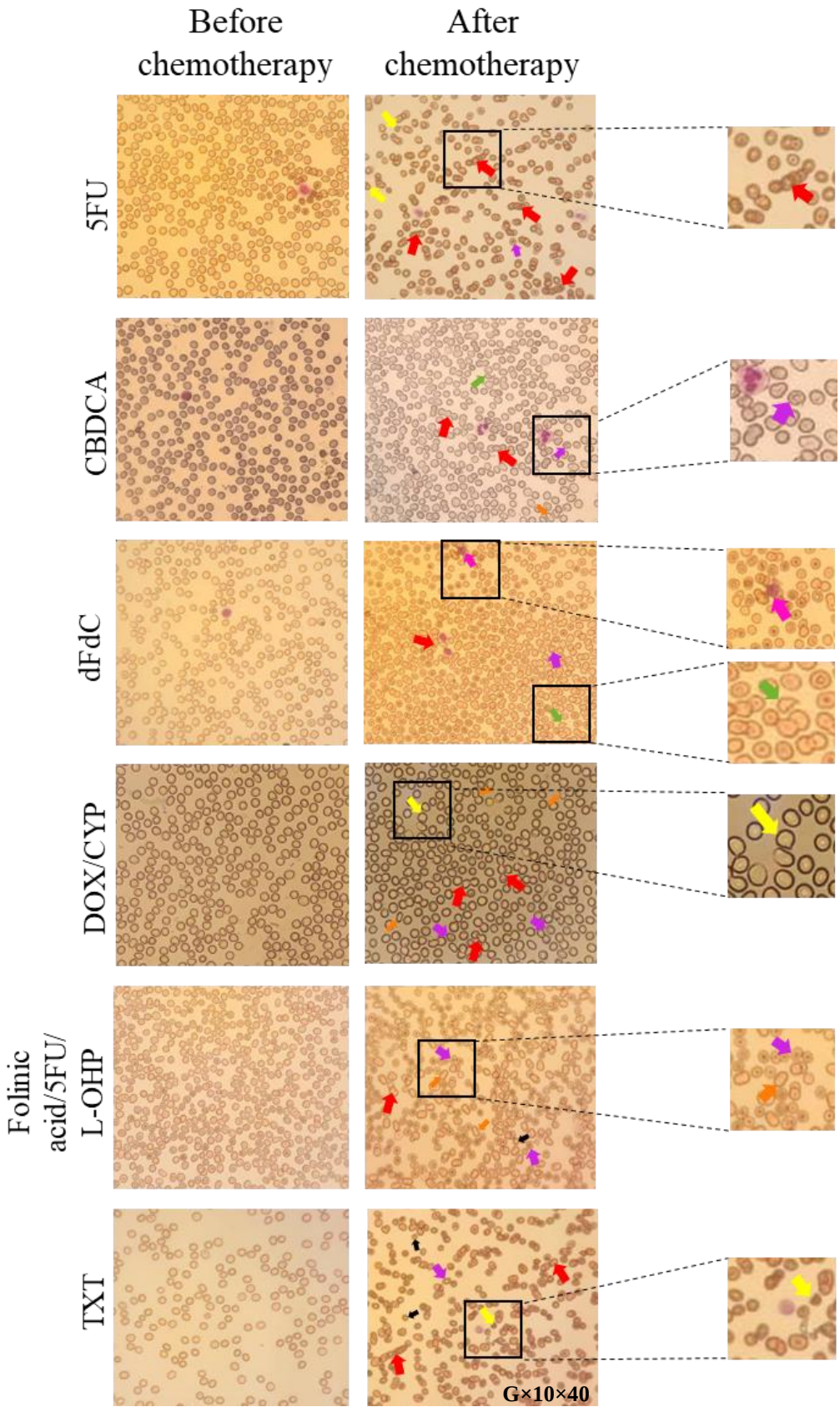

Figure 19: Peripheral blood smear before and after chemotherapy (G×10×40). Red arrow: rouleaux formation; yellow arrow: dacrocyte (teardrop cell); purple arrow: stomatocyte; green arrow: schistocyte; orange arrow: elliptocyte; black arrow: microcyte; pink arrow: lysed lymphocyte.

IV.1.3. Oxidative stress assay

- **Malondialdehyde (MDA) measurement results**

The results shown in **Figure 20** indicate MDA levels in pellets before and after chemotherapy. A slight increase was observed after the administration of 5FU and the combination of folinic acid/5FU/L-OHP and TXT (0.051±0.021; 0.035±0.03 and 0.077±0.039 respectively) compared to before receiving treatment (0.047±0.035, 0.028±0.03 and 0.020±0.008 respectively). In contrast, CBDCA (p=0.0075), and the combination of DOX/CYP (p<0.0001) resulted in a significant decrease in MDA levels (0.043±0.02 and 1.161±0.04, respectively) compared to before receiving treatment (0.160±0.034 and 1.202±0.102, respectively) while no significativity was observed after admistration dFdC (0.140±0.026) versus before chemotherapy (0.163±0.036).

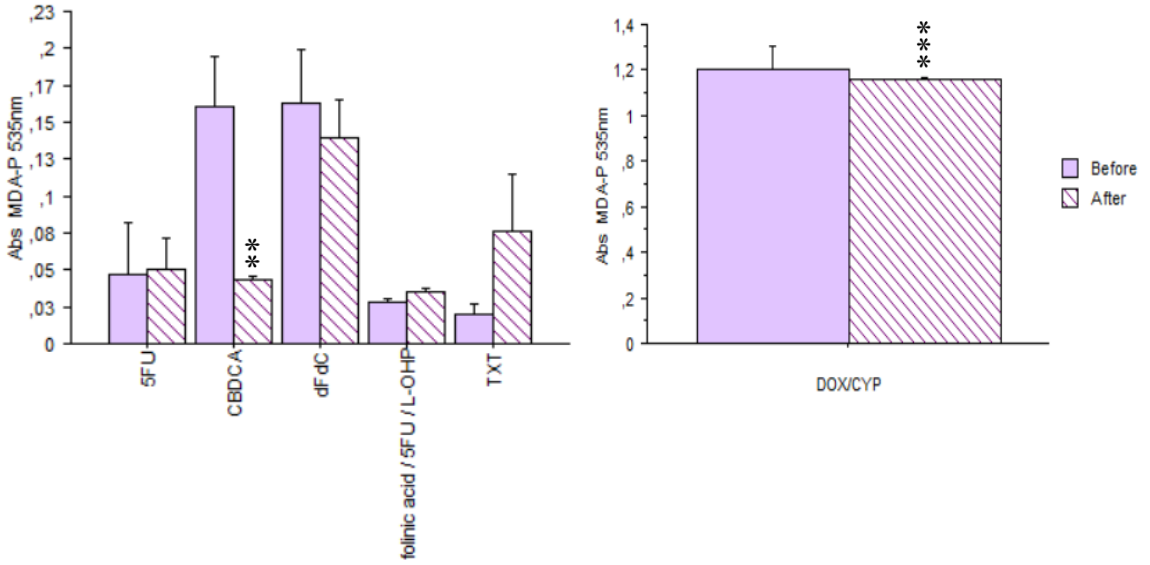

Figure 20: Pellets MDA levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

Figure 21 illustrates MDA levels in serum before and after chemotherapy. The results registered a modest elevation of MDA levels after the administration of 5FU, CBDCA, folinic acid/5FU/L-OHP polytherapy and TXT (0.100±0.007; 0.100±0.008; 0.045±0.024, and 0.051±0.044 respectively) compared to before treatment (0.060±0.013; 0.01±0.01; 0.026±0.019, and 0.049±0.026, respectively), while the administration of dFdC showed an higherelevation of MDA levels (1.161±0.673) versus (0.934±0.479) before treatment. In contrast, the regimen of DOX/CYP induces a slight decrease in MDA levels (1.065±0.09) when compared to before treatment (1.074±0.06).

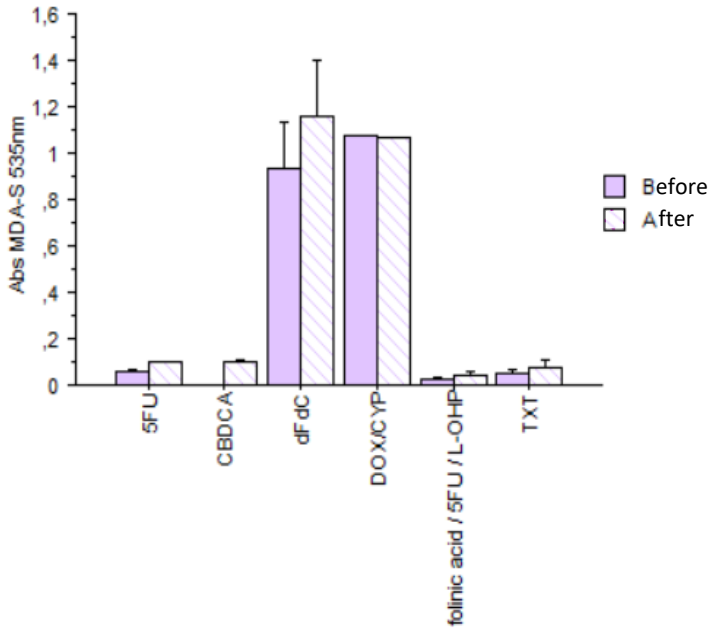

Figure 21: Serum MDA levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorobucin (DOX), cyclophosphamid (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

- **Results of hemoglobin and methemoglobin measurment**

Figure 22 highlights hemoglobin levels in pellets before and after chemotherapy. The administration of 5FU, dFdC, the combination of DOX/CYP and TXT registered a decrease in hemoglobin levels (0.535±0.668; 1.148±0.415; 1.257±0.230, and 0.205±0.122, respectively) comparing to before chemotherapy (0.729±430; 1.428±384; 1.534±0471, and 0.982±0.544, respectively) also a slight decrease was observed after administration of the polytherapy folinic acid/5FU/L-OHP (1.174±1.059) versus (1.181±0.223) before the treatment. Notably different, CBDCA showed an elevation in hemoglobin levels after treatment (1.791±0.00) compared to the results obtained before chemotherapy (1.666±0.00).

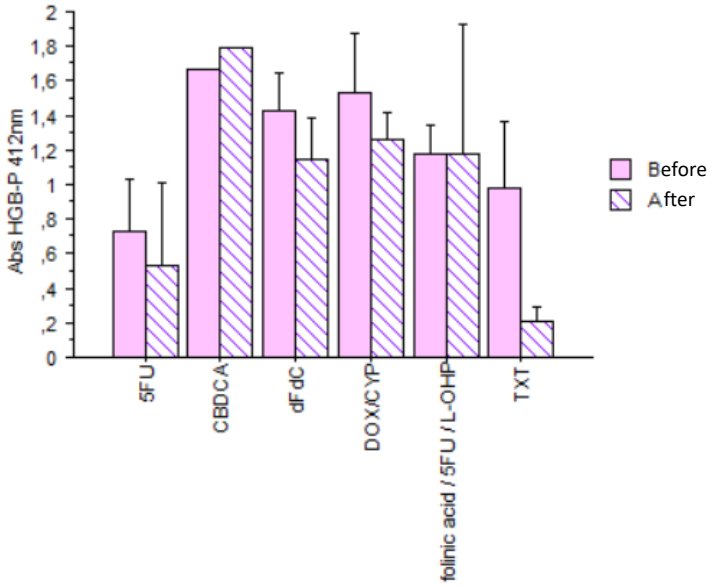

Figure 22: Pellets hemoglobin levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

Figure 23 represents hemoglobin levels in serum before and after chemotherapy. The treatment with 5FU and CBDCA resulted in a significant decrease in hemoglobin levels (0.279±0.164 and 0.339±0.00, respectively) compared to before treatment (0.610±0.121 and 0.793±0.00, respectively), while dFdC, the combination of folinic acid/5FU/L-OHP and TXT noted a slight decrease (0.272±0.088; 0.378±0.025, and 0.565±0.137, respectively) compared to the results obtained before receiving the treatment (0.309±0.105; 0.385±0.134, and 0.828±0.224, respectively). In contrast, the combination of DOX/CYP resulted in a high elevation of hemoglobin levels (1.534±0.419) versus (1.238±0.751) before treatment.

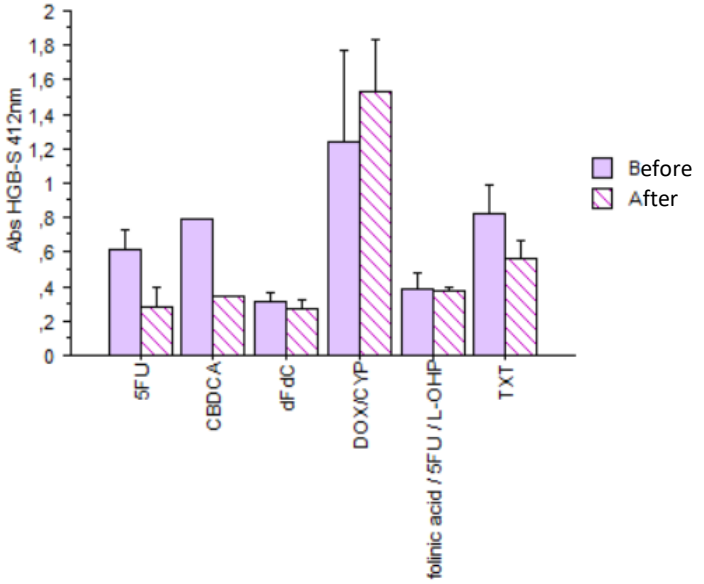

Figure 23: Serum hemoglobin levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

Methemoglobin levels in pellets before and after chemotherapy are shown in **Figure 24.** An important increase in MetHb levels was observed after the administration of 5FU, carboplatin, gemcitabine, the combination of folinic acid/5FU/L-OHP, and TXT (0.215±0.04; 0.241±0.00; 0.155±0.011; 0.192±0.056; and 0.122±0.051, respectively) compared to before treatment (0.182±0.086; 0.226±0.00; 0.134±0.01; 0.145±0.044; and 0.093±0.034, respectively). Meanwhile, the regimen combining DOX/CYP showed a slight elevation after treatment (0.178±0.068) compared to before the cure (0.175±0.069).

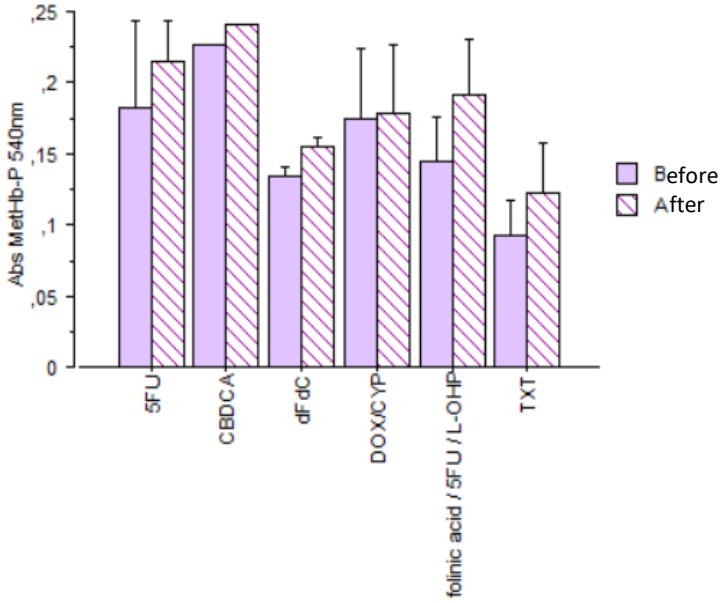

Figure 24: Pellets methemoglobin levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

Figure 25 illustrates methemoglobin levels in serum before and after chemotherapy. The results showed that the treatment with 5FU, dFdC, the combination of folinic acid/5FU/L-OHP and TXT led to an increase in MetHb levels (0.275±0.143; 0.075±0.028; 0.132±0.041, and 0.184±0.181, respectively) compared with before chemotherapy (0.217±0.093; 0.038±0.033; 0.077±0.026, and 0.168±0.087, respectively). In contrast, CBDCA and the combination of DOX/CYP showed a remarkable decrease in MetHb levels (0.080±0.00 and 0.320±0.161, respectively) compared to before treatment (0.136±0.00 and 0.372±0.165, respectively).

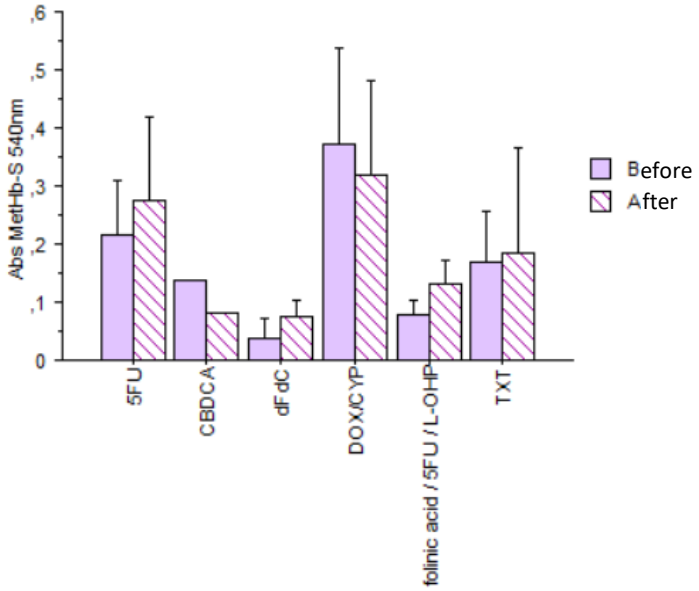

Figure 25: Serum methemoglobin levels before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorobucin (DOX), cyclophosphamid (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

IV.1.4. results of total antioxidant capacity measurment

The total antioxidant capacity (TAC) in pellets before and after chemotherapy is shown in **Figure 26.** The results indicated that TAC decreases after receiving 5FU, CBDCA, dFdC and combination of DOX/CYP (67.483±17.405%; 49.065±0.205%; 53.597±9.336% and 60.505±4.043% respectively) compared to before treatment (71.51±18.359%; 57.485±0.304%; 60.286±5.89%, and 67.95±8.84%, respectively). In contrast, an increase of TAC was observed after treatment with polytherapy folinic acid/5FU/L-OHP and monotherapy TXT (70.818±8.702% and 59.807±16.28%, respectively) compared to before treatment (56.892±3.533% and 54.723±22.175%, respectively).

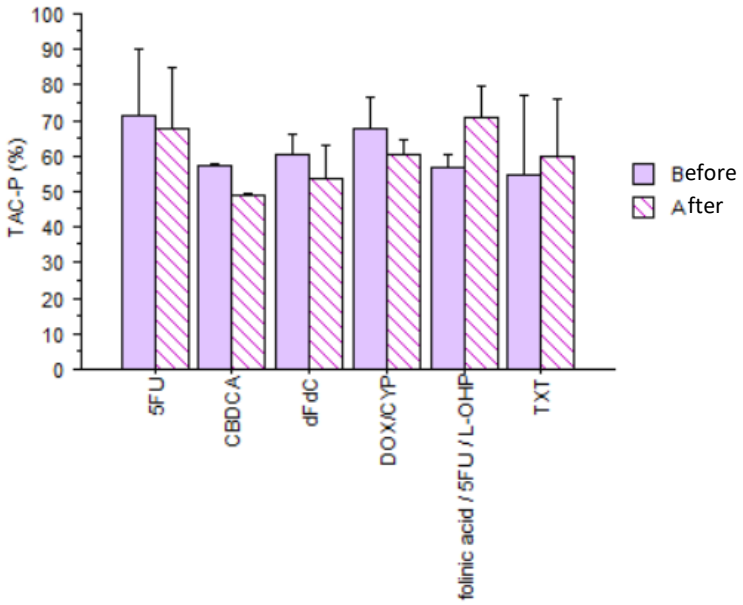

Figure 26: Pellets total antioxidant capacity before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

The results in **Figure 27** illustrate TAC in serum. It was observed that treatment with doxorubicin/cyclophosphamide resulted in a decrease in TAC (24.5±9.706%) compared to before the treatment (28.168±11.411%). However, an important increase in TAC was registered after the administration of CBDCA and the combination of folinic acid/5FU/L-OHP (28.63±3.055% and 25.93±6.37%, respectively) compared to the results obtained before chemotherapy (16.69±1.216% and 19.80±7.902%). In addition, a slight increase was observed in 5FU, dFdC and TXT (27.913±16.807% ;16.733±1.771% and 22.41±3.214% respectively) versus (27.663±11.068%; 16.427±2.717%, and 19.96±2.059%, respectively) before the treatment.

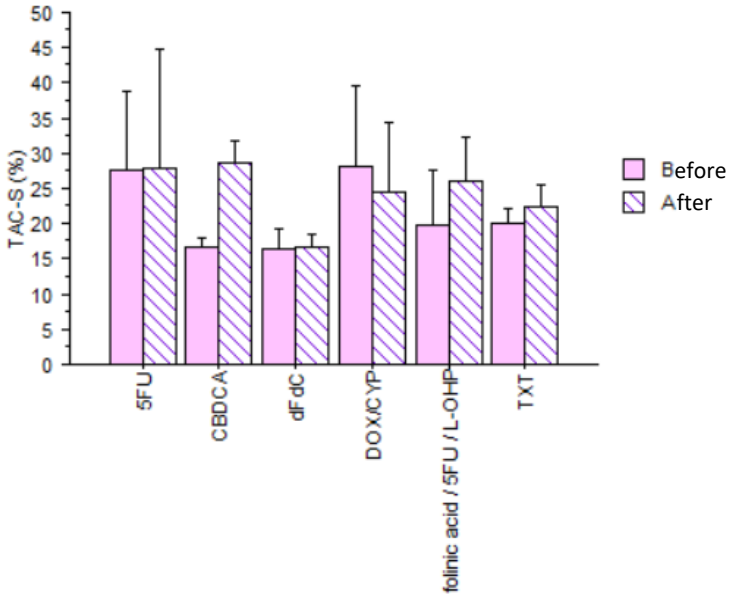

Figure 27: Serum total antioxidant capacity before and after chemotherapy. 5-fluorouracil (5FU), carboplatin (CBDCA), gemcitabine (dFdC), doxorubicin (DOX), cyclophosphamide (CYP), oxaliplatin (L-OHP), docetaxel (TXT). *p < 0.05; **p < 0.01; ***p < 0.001.

IV.2. Discussion

Chemotherapy is a treatment with high cytotoxicity, which often causes hematological disorders in patients who receive antineoplastic agents. These effects can lead to anemia, infections, and bleeding **(Yousaf et al., 2024).** The myelosuppressive effect is widely studied in the literature. However, the direct hemotoxic effect of chemotherapy drugs on whole blood cells in clinical trials remains under investigation.

This study aimed to assess the direct effect of chemotherapy on the hematological profile by comparing whole blood counts and blood smears; and to investigate the underlying mechanism of this hematotoxicity by measuring oxidative stress markers (MDA levels, hemoglobin and methemoglobin levels and total antioxidant capacity) before and after treatment.

The findings revealed that chemotherapy significantly decreased the hematological profile. It is shown that WBC count decreased after treatment with 5FU, CBDCA, dFdC, and TXT. The reduction of the WBC parameter can be related to the direct cytotoxic effect of chemotherapeutic agents. It has been reported by **Khan et al., (2022)** that the WBC count decreased after four chemotherapy cycles.

In fact, lymphopenia was observed after the administration of 5FU, dFdC, the combination of DOX/CYP, and folinic acid/5FU/L-OHP. This diminution may be due to the direct hematotoxic effect of the treatment. This finding is similar to an *in vivo* study conducted

by **Stahnke et al., (2001)** on 16 children's lymphocytes, which showed that T and B mature lymphocyte populations were reduced within 72h of treatment, primarily through apoptosis.

However, an increase in WBC was observed following treatment with the combination of DOX/CYP and folinic acid/5FU/L-OHP. At the same time, an elevation in lymphocyte count occurred after docetaxel treatment. This apparent elevation may be attributed to RBC aggregation observed on blood smears.

Indeed, RBC depletion was observed after the administration of 5FU, dFdC, and TXT. **Kassie et al. (2025)** found that RBC count decreased after chemotherapy for patients with sarcoma and lymphoma. Additionally, **Wondimneh et al. (2021)** found that RBC decreased significantly after chemotherapy due to ineffective erythropoiesis. This occurs because chemotherapy's nephrotoxic effects lead to a shortage of erythropoietin, a cytokine produced by the kidneys that stimulates erythropoiesis. Moreover, **Mameri et al. (2021)** showed, *in vitro*, that chemotherapy induced membrane cytotoxicity and hemolysis in healthy human RBC after a direct effect with different chemotherapeutic agents. It has been reported by **Skverchinskaya et al. (2023)** that incubating RBC with TXT and CBDCA caused swelling and hemolysis. Significant thrombopenia was observed following treatment with 5FU, DOX/CYP, and TXT, likely due to their direct cytotoxic effect. **Khan et al. (2022)** reported thrombopenia after four chemotherapy cycles, while **Wondimneh et al. (2021)** attributed platelet reduction to the destruction of early-stage megakaryocytic progenitors, which occurred even after a single treatment cycle.

Conversely, an increase in RBC was observed after the administration of CBDCA, the combination of DOX/CYP, and TXT. This elevation could be attributed to dehydration-induced hemoconcentration resulting from fluid loss or the mobilization of non-circulating erythrocytes into the bloodstream. However, this effect is transient and typically resolves quickly **(Carter, 2018).** Furthermore, this effect may be attributed to the cytotoxic effects of this drug on red blood cells, resulting in their aggregation. An *in vitro* study revealed that cisplatin induces a direct positive microrheological effect, potentially through interaction with specific molecular sites on erythrocyte membranes, thereby promoting RBC aggregation **(Muravyov et al., 2016).** These experimental findings align closely with our current observations.

Additionally, an overestimation of platelets was observed after treatment with dFdC and the folinic acid /5FU/L-OHP combination. In the case of dFdC, this elevation may be due to hemolysis caused by reduced RBC levels, leading to cellular debris being mistakenly counted as platelets by the automated analyzer. Also, it was reported that the magnitude of thrombocytosis in patients with cervical cancer is 27.9% **(Berta et al., 2024)**. Moreover,

Aynalem et al., (2022) found that the prevalence of thrombocytosis in breast cancer patients during and after treatment was 23.3% and 10.3% respectively.

Peripheral blood smears revealed significant morphological changes in the blood cells of cancer patients. Those treated with antineoplastic agents showed anisopoikilocytosis. Rouleaux formation and stomatocytosis were the most abnormal shapes observed on the smears. **Skverchinskaya et al., (2023)** showed in an *in vitro* study that the co-incubation of RBC with paclitaxel, CBDCA, dFdC and CYP fragilized the erythrocytes. Treatment with dFdC induced schistocytosis, which aligns with **Lee et al., (2014)** findings. The peripheral blood smear of a patient diagnosed with pancreatic cancer and treated with dFdC showed anisopoikilocytosis with schistocytes. In addition, **Skverchinskaya et al., (2023)** reported that incubating RBC with paclitaxel, which belongs to the taxane family, induced stomatocytosis. Also, the combination of paclitaxel/ CBDCA caused an additional poikilocytosis. As well, DOX/CYP incubation with RBC triggered elliptocytosis, echinocytosis, and schistocytosis. **Obama et al., (2023)** indicated that dFdC, CYP, and vincristine resulted in enhancing morphological abnormalities in RBC, including elliptocytes, anisocytosis, and schistocytes.

The lymphocyte destruction observed after receiving dFdC may be explained by the activation of apoptosis after exposure to OS, which is exacerbated by a high level of ROS. The study showed an increase in MDA levels in the pellets after treatment with 5FU, the combination of folinic acid /5FU/L-OHP, and TXT, which may be due to the lipid peroxidation of the RBC membrane phospholipids. This result is consistent with a recent study, which reported that MDA is an indicator of lipid peroxidation. This compound modifies the physiological properties of RBC membranes by inducing depolarization, disrupting protein transport, and inhibiting membrane enzymes **(Mameri et al., 2021).** Also, it was reported that paclitaxel increased lipid peroxidation as well as the rate of TBARS after DOX/paclitaxel infusion **(Panis et al., 2012).** In contrast, CBDCA, dFdC, and DOX/CYP combination showed a diminution of MDA levels in pellets, which may be due to a rapid intracellular antioxidant response.

The study shows an increase in MDA levels in the serum of patients treated with 5FU, CBDCA, dFdC, the combination of folinic acid/5FU/L-OHP, and TXT, which may be due to the release of the lipid peroxidation product (MDA), a small and water-soluble compound **(Tsikas, 2016).** On the other hand, a decrease in MDA levels was observed after treatment with the combination of DOX/CYP, possibly due to the activation of the plasma antioxidant system, which neutralizes oxidized lipids.

The results demonstrate a reduction in HGB levels in the pellets after the administration of 5FU, dFdC, the combination of DOX/CYP, TXT, and the polytherapy folinic acid/5FU/L-OHP, this may result from the ability of the chemotherapeutic agents to amplify and propagate OS within RBC, which is confirmed by **Mameri et al., (2021).**

The results showed a decrease in HGB in serum after treatment with 5FU, CBDCA, dFdC, folinic acid/5FU/L-OHP combination, and TXT. This reduction may result from chemotherapy's direct cytotoxic effect, potentially exacerbated by ROS-mediated HGB damage, leading to structural alterations and denaturation. In contrast, the elevation of HGB after the administration of DOX/CYP combination may be due to the release of HGB after cell lysis. These findings align with previous reports conducted by **Mameri et al., (2021).** Moreover, **Panis et al., (2012)** demonstrated that chemotherapy regimens consistently reduce hemoglobin levels, thereby exacerbating anemia in treated patients.

Our study also showed an increase in MetHb levels in the pellets after treatment with 5FU, CBDCA, dFdC, the combinations of DOX/CYP, folinic acid/5FU/L-OHP, and TXT. This effect may be due to the oxidation of HGB by ROS produced by these drugs. **According to Mameri et al., (2021)**, anticancer drugs can trigger the lysis of RBCs, causing the release of free HGB. This HGB then dissociates into alpha and beta dimers, which are later oxidized into MetHb-Fe3+.

The findings showed an elevation in MetHb levels in serum after the administration of 5FU, dFdC, the combination of folinic acid/5FU/L-OHP, and TXT, which may be due to the perturbation of the RBC membrane by reactive MetHb and its release into serum after hemolysis. In contrast, the reduction of MetHb after administration of CBDCA and the combination of DOX/CYP may be due to protein denaturation triggered by high ROS levels.

The total antioxidant capacity (TAC) test in pellets revealed a decrease after the administration of 5FU, CBDCA, dFdC, and a combination of DOX/CYP. This could be explained by the high level of OS affecting the cellular antioxidant system. **Panis et al., (2012)** demonstrated that patients treated with DOX showed reduced levels of glutathion (GSH) and total antioxidant capacity of plasma (TRAP).

In contrast, the elevation of TAC was observed after administering the regimen of folinic acid/5FU/L-OHP and TXT. This elevation may result from the rapid activation of cellular defense mechanisms in response to ROS damage, **Karkhanei et al., (2021)** found that TAC increases sharply in COVID-19 patients in intensive unit care which is a response to the oxidative stress triggered by the viral infection.

Analyses of TAC in serum showed a decrease after the administration of a regimen combining DOX/CYP. This may be due to alterations in the antioxidant system in serum, which is consistent with the findings of **Panis et al., (2012).** While the elevation of TAC in serum after receiving 5FU, CBDCA, dFdC, the combination of folinic acid/5FU/L-OHP, and TXT was observed; it can be related to the activation of the antioxidant system in plasma in response to ROS attack.

Conclusion

Conclusion

Chemotherapy is the most common treatment for cancer by inducing cell death. However, it also affects healthy cells, causing several toxicities such as hematotoxicity exacerbated by oxidative stress, including anemia, thrombopenia, and leukopenia.

In this study, we conducted blood cell counts on cancer patients before and after chemotherapy. Our results indicate that anti-cancer drugs induced a direct cytotoxic effect on blood cells. The results were also confirmed by blood smears, which revealed cellular morphological abnormalities after treatment.

Oxidative stress (OS) assays were assessed as an underlying mechanism of this toxicity, including lipid peroxidation by measuring MDA levels, MetHb generation, and TAC. The antineoplastic agents induced OS through lipid peroxidation and enhanced MetHb formation, which are harmful to blood cells. The TAC test was performed by measuring the neutralization of ABTS radical. It was found that chemotherapy negatively affects the antioxidant system, but cellular antioxidant defenses may be activated after treatment administration as an adaptative response to ROS attack.

More investigations are required to identify the molecular mechanism involved in this toxicity to prevent hematotoxicity in cancer patients. It will be essential to expand this research to include a greater number of patients. This would also enable a more accurate determination of the ideal antioxidant to combine with anticancer treatments, thereby helping physicians tailor supportive care strategies to mitigate side effects and improve patients' quality of life.

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Annexe

Consent

Je soussigné Mr/Mme :

Né le : à donne par la présente mon consentement pour le prélèvement et l'utilisation de mon sang avant et après la chimiothérapie dans le cadre de la recherche scientifique. Ce prélèvement sera effectué après l'accord de mon médecin traitant, Pr. Mazouzi.

Je comprends que toutes les données obtenues à partir de mon sang seront anonymisées. Cela signifie que mes informations personnelles telles que mon nom, mon adresse et toute autre donnée d'identification ne seront pas associées à mes échantillons sanguins dans les analyses et les rapports résultants.

Signature de médecin traitant Signature de patient

Abstract: Chemotherapy is the most common treatment for cancer. However, it causes several side effects, including hematotoxicity. This study aimed to investigate the direct impact of chemotherapy on cancer patients' blood cells, focusing on three principal axes: hematological, morphological, and biochemical, including the exploration of oxidative stress (OS) in this hematotoxicity. Blood cell count results showed a decrease in hematological profile after chemotherapy notably in patients treated with 5FU. Moreover, peripheral blood smears revealed anisopoikilocytosis after the administration of all chemotherapeutic agents, highlighting the high hematotoxicity manifested by morpho-abnormalities in blood cells. Furthermore, OS assay showed that TXT increased MDA levels in pellets after treatment (0.077±0.039) compared to before treatment (0.020±0.008). dFdC also caused an increase in MDA levels in serum (1.161±0.673) versus (0.934±0.479) after chemotherapy. Hemoglobin measurement showed a significant decrease in pellets after receiving TXT (0.982±0.544) versus (0.205±0.122) and after receiving CBDCA, the HGB level in serum was (0.793±0.00) versus (0.339±0.00) after chemotherapy. An elevation of MetHb in pellets after the administration of all chemotherapeutic agents was observed, notably the combination of folinic acid/5FU/L-OHP (0.145±0.044) versus (0.192±0.056). In addition, CBDCA showed a decrease in TAC in pellets (57.485±0.304%) versus (49.065±0.205%), as well as in serum of patients treated with DOX/CYP (28.168±11.411%) versus (24.5±9.706%). Antineoplastic agents induce notable damages in blood cells, including morpho-abnormalities and redox imbalance. Further studies are required to understand the underlying molecular mechanism involved. These findings may serve as a conceptual baseline for developing strategies to prevent hematotoxicity and improve treatment outcomes.

Keywords: Chemotherapy, hematotoxicity, oxidative stress, lipid peroxidation, blood smear.

Résumé : La chimiothérapie demeure le traitement de choix contre le cancer en raison de sa capacité à induire la lyse des cellules tumorales. Cependant, elle est souvent associée à de nombreux effets indésirables notamment l'hématotoxicité. Cette étude a pour objectif d'évaluer l'effet direct de la chimiothérapie sur les cellules sanguines de patients cancéreux, structurée selon trois axes principaux : hématologique, morphologique et biochimique en explorant le rôle du stress oxydant dans cette hématotoxicité. Les résultats de l'hémogramme ont révélé une diminution du profil hématologiques particulièrement chez les patients traités avec le 5FU. Les frottis sanguins périphériques ont montré une anisopoikilocytose après l'administration de l'ensemble des agents de chimiothérapie mettant en évidence les altérations morphologiques des cellules sanguines. Les dosages des marqueurs de SO ont révélé que le TXT induisait une augmentation des taux de MDA dans les culots cellulaires (0.077±0.039) contre (0.020±0.008), tandis que dFdC induisaitt une augmentation des taux des MDA dans le sérum du (0.934±0.479) à (1.161±0.673). Les concentrations d'hémoglobine ont nettement diminué dans les culots après le traitement par le TXT de (0.982±0.544) à (0.205±0.122) et dans le sérum après administration de CBDCA de (0.793±0.00) à (0.339±0.00). Une élévation des taux de MetHb a également été observée dans les culots cellulaires après l'administration de l'ensemble des agents de chimiothérapie, en particulier après la polythérapie d'acide folinique/5FU/L-OHP de (0.145±0.044) à (0.192±0.056). En outre, le CBDCA a induit une diminution de la capacité antioxydante totale dans les culots de (57.485±0.304%) à (49.065±0.205%) ainsi qu'une baisse de cette capacité dans les sérums des patients traités par DOX/CYP (24.5±9.706%) contre (28.168±11.411%) avant le traitement. Les agents antinéoplasiques induisent des altérations importantes aux cellules sanguines incluant des anomalies morphologiques et un déséquilibre redox. Des recherches supplémentaires sont nécessaires pour élucider les voies moléculaires sous-jacentes. Ces résultats pourraient constituer une base conceptuelle pour le développement de stratégies visant à prévenir l'hématotoxicité pour améliorer l'efficacité des traitements anticancéreux.

Mots clés : hémototoxicité, chimiothérapie, stress oxydant, peroxydation lipidique, frottis sanguin.

ملخص : تُعدُّ العلاج الكيميائي الخيار العلاجي الأساسي في مواجهة السرطان نظرًا لقدرتها على تحفيز تحلل الخلايا الورمية. ومع ذلك، غالبًا ما يرتبط العلاج الكيميائي بعدد كبير من التأثيرات الجانبية، لا سيما السُمِّيَّة الدموية. تهدف هذه الدراسة إلى تقييم التأثير المباشر للعلاج الكيميائي على خلايا الدم لدى المرضى المصابين بالسرطان، من خلال ثلاثة محاور رئيسية: الدموي، والمورفولوجي، والبيوكيميائي، مع التركيز على دور الإجهاد التأكسدي في هذه السُمِّيَّة الدموية .كشفت نتائج تعداد الدم عن انخفاض في المؤشرات الدموية، خاصة لدى المرضى الذين عُولجوا بـ 5 .FU-كما أظهرت لطاخات الدم المحيطي وجود أنيسوبويكيلوسيتوز (اختلاف في شكل وحجم خلايا الدم الحمراء) بعد إعطاء جميع العوامل الكيميائية، مما يدل على تغيرات مورفولوجية في خلايا الدم .أظهرت قياسات مؤشرات الإجهاد التأكسدي أن دواء TXT سبّب ارتفاعًا في مستويات MDA في كُتل الخلايا (0.077±0.039) مقارنة بـ (0.020±0.008)، بينما أدّى دواء dFdC إلى زيادة مستويات MDAفي المصل من (0.934±0.479) إلى .(1.161±0.673) كما لوحظ انخفاض واضح في تركيزات الهيموغلوبين في الكُتل الخلوية بعد العلاج بـ TXT من (0.982±0.544) إلى (0.205±0.122)، وفي المصل بعد إعطاء CBDCA من (0.793±0.00) إلى .(0.339±0.00) كما تم رصد ارتفاع في مستويات الميتهيموغلوبين (MetHb) في الكُتل الخلوية بعد إعطاء جميع عوامل العلاج الكيميائي، خصوصًا بعد العلاج المشترك بالفولينات / 5 L-OHP / FU-من (0.145±0.044) إلى .(0.192±0.056) علاوة على ذلك، أدّى دواء CBDCA إلى انخفاض في القدرة المضادة للأكسدة الكلية في الكُتل من (57.485±0.304%) إلى (49.065±0.205%)، إضافة إلى انخفاض هذه القدرة في أمصال المرضى الذين عولجوا بـ DOX/CYP من (28.168±11.411%) إلى (24.5±9.706%) بعد العلاج .تُحدث العوامل المضادة للسرطان تغيّرات كبيرة في خلايا الدم، بما في ذلك تشوهات مورفولوجية واختلال في التوازن التأكسدي. لا بد من إجراء المزيد من الأبحاث لتوضيح المسارات الجزيئية الكامنة وراء هذه التأثيرات. وقد تُشكل هذه النتائج قاعدة مفهومية لتطوير استراتيجيات تهدف إلى الوقاية من السُمِّيَّة الدموية، وبالتالي تحسين فعالية العلاجات المضادة للسرطان.

الكلمات المفتاحية: السمية الدموية، العلاج الكيميائي، الإجهاد التأكسدي، تأكسد الدهون، اللطاخة الدموية.