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AN *IN VITRO* INVESTIGATION OF THE INTERACTION OF GENOMIC DNA WITH SOME COPPER COMPOUNDS

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DEPARTMENT OF BIOCHEMISTRY (VETERINARY)

MASTER'S THESIS

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ACCEPTANCE and CONFIRMATION

The research thesis entitled "AN IN VITRO INVESTIGATION OF THE INTERACTION OF GENOMIC DNA WITH SOME COPPER COMPOUNDS" presented by Elisha Apatewen AKANBONG has been accepted and approved by the jury members as a MASTER'S THESIS in the Department of Biochemistry (Veterinary), Kırıkkale University.

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I affirm the scope and quality of this thesis as a Master's Thesis. ..……

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Date of Defence: 16/May/2022

I accept and affirm that this thesis accepted and approved by the jury members meets the standards and conditions of a Master's Thesis.

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ABSTRACT

Genomic DNA constitutes the total genetic information of an organism and it is the basis for processes necessary for life such as transcription, recombination, cell survival, and proliferation. Under normal physiological conditions, cations such as K^+, Na^+, and Mg^+ contribute to the normal functioning and stability of genomic DNA through electrostatic interaction. Although the roles of metal ions in protecting DNA stability and structure are known, some of them can be mutagenic and carcinogenic. In living organisms, transition metals that interact with DNA above physiological limits can lead to irreversible cell damage and ultimately cell death.

On the other hand, the number of scientific studies aiming to discover new therapeutic agents that change or inhibit the activity of genomic DNA, especially anti-cancer agents, is increasing day-by-day. The bonds it forms with chemical agents in its environment critically affect its activity and investigating the interaction of DNA with metal compounds contributes to the design of effective therapeutic agents and better and safer anti-cancer drugs.

In this study, to investigate the interaction of genomic DNA with some copper compounds ($CuSO_4$, $CuCO_3$, and $CuCl_2$) at various concentrations (1000 μM, 500 μM, 250 μM, 125 μM, and 62.5 μM); UV-VIS absorbance spectrophotometry, agarose gel electrophoresis and fluorescence spectrophotometry techniques were used.

When the UV-VIS spectrophotometry data were examined, the hyperchromic effect of $CuCl_2$ was evaluated to be proportional to its concentrations, within the absorbance range of 220-320 nm. $CuCl_2$ had the strongest interaction with DNA at 1000 μM. Within the same absorbance range, $CuSO_4$ and $CuCO_3$ also produced their strongest hyperchromic effect on the DNA at 1000 μM. When the fluorescent spectrophotometry

data were evaluated, the hypochromic effect of $CuCl_2$ within the wavelength range of 400-700 nm was determined at 1000 µM. $CuSO_4$ showed a hypochromic effect (400-700 nm) at concentrations above 62.5 µM. However, this effect was not proportional to its concentrations, and the strongest hypochromic effect occurred at 125 µM. Upon evaluating the fluorescent spectrophotometry data, it was observed that $CuCO_3$ did not interact with DNA at the concentrations examined within the scope of this study. According to the agarose gel electrophoresis findings, all the three copper compounds investigated caused a decrease in DNA band intensities as their concentrations increased. It was observed that the most significant decrease in band intensities was caused by $CuCl_2$ at 1000 µM. When the agarose gel electrophoresis images were examined, it was observed that the copper compounds within the scope of the study did not cause any cleavage in the genomic DNA within the concentration range 62.5-1000 µM.

After evaluating the results of the study, it was concluded that the investigated copper compounds bind to DNA via non-intercalative (groove) binding. This result reveals that the investigated copper compounds may have the potential to be used in the development of new therapeutic agents. In conclusion, the potential effect detected could form the basis of new research and it would be beneficial to investigate the effect of these compounds on cancer cell lines.

Keywords: Genomic DNA, Copper Compounds, DNA-Metal Interaction

ÖZET

Genomik DNA, bir organizmanın toplam genetik bilgisini oluşturmakta olup, transkripsiyon, rekombinasyon, hücre sağ kalımı ve çoğalması gibi canlılık için gerekli süreçlere temel teşkil eder. Normal fizyolojik koşullar altında, K^+, Na^+ ve Mg^+ gibi katyonlar, elektrostatik etkileşim yoluyla genomik DNA'nın normal işleyişine ve stabilitesine katkıda bulunurlar. Metal iyonlarının, DNA stabilitesini ve yapısını koruma rolleri bilinmekle birlikte, bazıları ise mutajen ve kanserojen etkili olabilmektedir. Canlı organizmalarda, DNA ile fizyolojik sınırların üzerinde etkileşime giren geçiş metalleri, geri dönüşü olmayan hücre hasarına ve nihayetinde hücre ölümüne yol açabilir.

Diğer yandan, başta anti-kanser ajanlar olmak üzere genomik DNA'nın aktivitesini değiştiren veya inhibe eden yeni terapötik ajanların keşfedilmesini amaçlayan bilimsel çalışmaların sayısı her geçen gün artmaktadır. Çevresindeki kimyasal ajanlarla kurduğu bağlar DNA'nın aktivitesini kritik bir şekilde etkilemekte olup, DNA'nın metal bileşikleriyle etkileşiminin araştırılması, etkili terapötik ajanların ve daha iyi ve güvenli anti-kanser ilaçların tasarlanmasına katkıda bulunmaktadır.

Bu çalışmada, genomik DNA'nın çeşitli konsantrasyonlardaki (1000 μM, 500 μM, 250 μM, 125 μM ve 62,5 μM) bazı bakır bileşikleri ($CuSO_4$, $CuCO_3$ ve $CuCl_2$) ile etkileşiminin araştırılması; UV-VIS absorbans spektrofotometri, agaroz jel elektroforezi ve floresan spektrofotometri teknikleri ile gerçekleştirildi. Araştırmamız kapsamında, söz konusu biyokimyasal analiz teknikleri kullanılarak yapılan tüm ölçümler, sabit genomik DNA ve değişken bakır bileşikleri konsantrasyonları üzerinde yapıldı. Bu çalışmada kullanılan genomik DNA, buzağı timüsünden izole edildi. İzole edilen ve 260/280 nm'de 1.84 saflığa sahip olan genomik DNA; UV-absorbans, agaroz jel elektroforezi ve floresan spektrofotometri çalışmalarında kullanıldı. DNA-bakır bileşiği karışımları, steril eppendorf tüplerinde 1:1 oranında (5 μl + 5 μl) hazırlandı. Bu işlemi takiben, tüm kombinasyonlar 37°C'de 60 dakika süreyle inkübe edildi. DNA, bakır bileşiği ve DNA+bakır bileşiği örneklerinin UV-absorbans ölçümleri spektrofotometre cihazı ile gerçekleştirildi. Cihaz kalibrasyonu ve ölçümlerde nükleaz içermeyen saf su tercih edildi. Floresan spektrofotometri çalışmalarında ise DNA, DNA boyası, bakır

bileşikleri ve DNA+bakır bileşiklerinin floresan yoğunluğu, çok modlu bir mikroplaka okuyucu ile 290 nm eksitasyon değerinde ve 400-700 nm emisyon aralığında ölçüldü. Agaroz jel elektroforezi için 10 µl DNA+bakır bileşiği 2 µl DNA yükleme boyası ve 2 µl DNA boyası kullanıldı. Yükleme işleminin ardından 100 voltta 1,5 saat elektroforez işlemi gerçekleştirildi.

UV-VIS spektrofotometri verileri incelendiğinde 220-320 nm absorbans aralığında konsantrasyon ile orantılı olmak üzere $CuCl_2$'ün hiperkromik etkisi belirlendi. $CuCl_2$ DNA ile en güçlü etkileşimi 220-320 nm absorbans aralığında 1000 µM dozda gerçekleştirdi. Aynı absorbans aralığında 1000 µM konsantrasyonda $CuSO_4$ ve $CuCO_3$ de DNA pikinde en büyük hiperkromik etkiyi oluşturdu. Floresan spektrofotometri verileri incelendiğinde bakır bileşiklerinden $CuCl_2$'nin 1000 µM konsantrasyonda 400-700 nm aralığında oluşan hipokromik etkisi belirlendi. $CuSO_4$ bileşiği ise 62,5 µM üzerindeki konsantrasyonlarda hipokromik etki (400-700 nm) gösterdi. Ancak bu etki derişimle orantılı olmayıp en güçlü hipokromik etki 125 µM $CuSO_4$ konsantrasyonunda ortaya çıktı. $CuCO_3$'ün floresan spektrofotometri verileri değerlendirildiğinde ise çalışmamız kapsamında incelenen konsantrasyonlarda DNA ile etkileşmediği belirlendi. Agaroz jel elektroforezi bulgularımız araştırılan üç bakır bileşiğinin de DNA bant yoğunluklarında konsantrasyon ile artış gösteren bir azalmaya sebebiyet verdiğini ortaya koydu. Bant yoğunluklarındaki en belirgin azalmaya (29,762) 1000 µM konsantrasyonundaki $CuCl_2$'nin sebep olduğu gözlendi. Yine 62,5 µM konsantrasyonundaki $CuCl_2$, 49,757 bant yoğunluğuna sahip kontrol DNA'sından sonra en yüksek bant yoğunluğunu (44,276) gösterdi. $CuSO_4$ da en düşük bant yoğunluğunu 1000 µM'de (36,414) ve en yüksek bant yoğunluğunu 62,5 µM'de (40,139) gösterdi. Aynı şekilde $CuCO_3$ 1000 µM'de en düşük bant yoğunluğunu (39,980) ve 62,5 µM'de en yüksek bant yoğunluğunu (42,979) gösterdi. Agaroz jel elektroforezi görüntüleri incelendiğinde araştırma kapsamındaki bakır bileşiklerinin 62,5-1000 µM konsantrasyon aralığında genomik DNA bantlarında herhangi bir bölünmeye neden olmadığı tespit edildi.

Mevcut çalışmanın sonuçları bir arada değerlendirildiğinde, araştırılan bakır bileşiklerinin, interkalatif olmayan (oluk) bağlanma yoluyla DNA'ya bağlandığı sonucuna ulaşılmıştır. Bu sonuç, araştırılan bakır bileşiklerinin yeni terapötik ajanların geliştirilmesinde kullanılma potansiyeline sahip olabileceğini ortaya koymaktadır. Tespit edilen potansiyel etkinin yeni araştırmalara temel oluşturabileceği ve bu bileşiklerin kanser hücre hatlarında araştırılmasının faydalı olacağı sonucuna varılmıştır.

Anahtar Kelimeler: Genomik DNA, Bakır Bileşikleri, DNA-Metal Etkileşimi

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LIST OF ACRONYMS AND SYMBOLS

DNA	Deoxyribose Nucleic Acid
ct-DNA	Calf thymus- Deoxyribose Nucleic Acid
RNA	Ribonucleic Acid
$CuCl_2$	Copper (II) Chloride
$CuCO_3$	Copper (II) Carbonate
$CuSO_4.5H_2O$	Copper (II) Sulphate pentahydrate
mM	Millimolar
UV	Ultraviolet
Cu	Copper
EB	Ethidium Bromide
ROS	Reactive Oxygen Species
MRI	Magnetic Resonance Imaging
µmol	Micromole
µl	Microlitre
µg	Microgram
mg	Milligram
OH^-	Hydroxyl
A	Adenine
T	Thymine
G	Guanine
C	Cytosine
nm	Nanometer
α	Alpha
β	Beta
°	Degree
%	Percentage
Å	Angstrom

ESI	Electrospray Ionization
MALDI	Matrix-Assisted Laser Desorption Ionization
HPLC	High-Performance Liquid Chromatography
LD	Linear Dichroism
CD	Circular Dichroism
*K*b	Intrinsic Binding Constant
EDTA	Ethylene Diamine Tetra-acetic
TBE	Tris-Boric acid- Ethylene Diamine Tetra-acetic
g	Gram
ml	Millilitre
IC_{50}	Half Maximal Inhibitory Concentration
℃	Degree Celsius
USA	United States of America
UK	United Kingdom

1. INTRODUCTION

1.1 Background

DNA is a nucleic acid, a genetic material that mediates crucial processes like; transcription, recombination, cell survival, and proliferation. Under normal physiological conditions, cations such as K^+, Na^+ , and Mg^+ contribute to the normal functioning, and stability of DNA through their electrostatic interactions with DNA (Williams and Da Silva, 2005). Some metal ions are also essential in the maintenance of DNA stability and structure, but some are also known to be mutagenic, and carcinogenic. Relevant facts about the mutagenicity, and carcinogenicity of metal ions were obtained from several research studies conducted on the interactions between d-block elements and DNA. In living organisms, non-physiological transition metals interacting with nucleic acids, DNA can lead to irreversible cell damage and eventually, apoptosis (Lippert, 2008). Modifying or inhibiting the activity of a cell's DNA is essential medical research that enhances the development of new therapeutic agents, mainly anticancer agents. DNA-targeting therapeutic agents are effective therapeutic agents, and some have significantly increased the survival of cancer patients when used in combination with other drugs having different mechanisms of action (Hurley, 2002). Among the therapeutic agents like the anticancer agents, DNA cleaving agents have attracted the attention of scientists in the field of molecular biology and drug development (Khan et al., 2017).

The vital role of metal ions in biology and medicine is a subject studied intensively across the globe (Hannon and Reedijk, 2015). A wide range of fields including material and biological sciences develop, and apply metal-containing compounds (Boerner and Zaleski, 2005; Zhang and Lippard, 2003). Due to the high biological interest in metal complexes, they are used widely in medicine as Magnetic Resonance Imaging (MRI) contrast agents, in radiopharmaceuticals, in the treatment

of ulcers, arthritis, and also in cancer chemotherapy (Kong et al., 2009; Storr et al., 2006; Thompson and Orvig, 2006). For instance, the chemotherapeutic treatment for different malignancies adopts the use of the platinum-based (heavy-metal) drug (cisplatin) (Hassan et al., 2010). Following the discovery of metal-based drugs such as cisplatin by Rosenberg and colleagues, there has been significant attention on understanding, and developing metal-based drugs. However, metal-based drugs like cisplatin have known toxic effects such as; neurotoxicity, myelotoxicity, nephrotoxicity, hepatotoxicity, and drug resistance (Bhattacharjee et al., 2021; Hassan et al., 2010). In response to these toxic effects, alternate metal complexes such as copper complexes are potential therapeutic candidates for medicinal research as these metal complexes can interact efficiently with DNA under physiological conditions. A tremendous effort has been made to develop several transition metal complexes including copper complexes as therapeutic agents (Bhattacharjee et al., 2021). Copper (Cu) is a versatile transition metal necessary for all life forms. Copper has a vast variety of functions ranging from angiogenesis, antioxidant, a moiety of plastocyanin (essential for photosynthesis in plants and algae), a moiety of hemocyanin in arthropods and gastropods blood, oxidative phosphorylation, connective tissues cross-linking, mobilization of iron, catecholamine synthesis, and as a cofactor for several enzymes including cytochrome oxidase, which is the terminal enzyme of the respiratory electron transport chain. (Robinson and Winge, 2010). Aside from being a component of some enzymes, it also forms an integral moiety of crucial proteins such as ceruloplasmin and albumin (Iakovidis et al., 2011). Its deficiency is accompanied by profound clinical outcomes which are often associated with neurodegeneration (Robinson and Winge, 2010).

Copper when ingested with food (organic copper), it is processed by the liver, transported, and sequestered safely. However, inorganic copper which is often ingested through drinking water and copper supplements largely enter the free blood copper pool directly by bypassing the liver. This copper is potentially hazardous as it may penetrate the blood-brain barrier. Generally, when copper is absorbed from the stomach and small intestine, it is transported to the liver via portal blood by bounding to albumin. It is then transported to peripheral tissues by ceruloplasmin and to a lesser extent, by albumin. The liver contains 10 % of the total body copper content, 1200 μmol (80 mg). The excess

copper is released into the gut in bile to be excreted out of the body (Iakovidis et al., 2011). Dietary intake of copper salts, which generally exceeds the demand by tissues and excretion of excess copper via bile enhances a proper balance of copper (homeostasis). However, an impairment in the biliary excretion of copper may cause the accumulation of copper in the liver and brain (Wilson's disease) (Robinson and Winge, 2010). Cu also elicits congenital effects (teratogenic effects), the induction of mutation via chromosome breakage (clastogenic effect), and spindle disturbances (Agarwal et al., 1989). Also, excess copper can cause jaundice. On the other hand, its deficiency can lead to the occurrence of abnormalities such as anaemia and hair kinky (Kocharekar and Thakkar, 2004).

Cu complexes have the aptitude for interacting with DNA due to their three-dimensional structure, cationic ability, the tendency of hydrolysing DNA, and their redox ability (Boerner and Zaleski, 2005). Copper's ability to accept and donate electrons makes it a good redox cofactor. But, also, a complicit of the Fenton's reaction thus, capable of driving the generation of reactive oxygen species (ROS). For instance; the use of copper chelators such as ligand-L (Coumarin) instigates DNA cleavage, oxidative DNA damage via the generation of the deleterious highly reactive oxygen, hydroxyl (OH^-) in the presence of Cu (II) as a result of ligand-L induced redox cycling of Cu (II) (Khan et al., 2017). Cu (II) complexes have been used as tools for the mediation of strand scission of duplex DNA. They have also been used as probes of DNA structure in the solution phase (Khan et al., 2017). Metal complexes including copper complexes have shown varying efficiencies in hydrolysing RNA and phosphate diester (Bhattacharjee et al., 2021; Bím et al., 2016; Tirel et al., 2014). In addition, Cu (II) and Cu (I) complexes made of naturally occurring and synthetic ligands can mimic the activity of nuclease. Some Cu (II) complexes have also exhibited the ability to mediate the cleavage of the double-strand of plasmid DNA non-randomly (Bencini et al., 2003; Sissi et al., 2005).

There is a recent increase in interest in research on nucleic acids interaction with transition metal ions as this contributes to the development of biotechnological tools and medicine. DNA binding critically influences the activity of DNA, hence, exploring the interaction of DNA with metal complexes will contribute to designing effective

therapeutic agents, better, and safer anticancer drugs (Bhattacharjee et al., 2021; Pages et al., 2015). In spite of this, the role of metal ions and metal complexes in binding with nucleic acids has been less studied (Hannon and Reedijk, 2015). In view of this, there is no, if any, little study on the interaction of $CuSO_4$, $CuCO_3$, and $CuCl_2$ with DNA. Thus, this study was aimed to investigate the interaction of DNA with $CuSO_4$, $CuCO_3$, and $CuCl_2$, to serve as potential candidates for drugs development.

1.2 History of DNA

During the second half of the 19th century, the focus of biologists shifted from the study of organisms, organs, and tissues to the study of cells. Matthias J. Schleiden and Theodor Schwann demonstrated that tissues were composed of cells and that it is these cells that interact to give rise to the complex animal or plant (Mayr, 1982). Louis Pasteur and Rudolph Virchow also rebutted the previous notion that life emerged from lifeless matter by demonstrating that new cells did not emerge from lifeless matter. They reported that new cells emerged from pre-existing cells (Maulitz, 1978). Subsequently, there was attention on hereditary and evolution, and this led to Charles R. Darwin and Alfred R. Wallace's theories of evolution by natural selection in 1858 (Darwin, 1859). In 1865, Gregor Mendel also proposed the laws of heredity from his breeding experiments with peas (Mendel, 1866), which in 1900 were rediscovered by Carl Correns (Correns, 1900), Hugo de Varies (De Vries, 1900a; De Vries, 1900b), and Erich von Tschermak (Sturtevant, 2001; Tschermak, 1900).

In line with various studies that were conducted on heredity, the observations made by researchers including; Theodor Boveri, Walther Flemming, Ernst Haeckel, and Edmund B. Wilson led to the merging of the two emerging fields (cytology and genetics), hence, the coining of the field cytogenetics. For instance; it was proposed by Haeckel in 1866 that the nucleus contained the factors necessary for hereditary traits transmission (Dahm, 2005). This finding incited an increase in research interest in the nucleus. Following the attention attracted by the nucleus, Flemming in 1879 conducted a study to describe the morphology and behaviour of chromosomes during mitosis and resulted in the coining of the terms "chromatin" and "mitosis" (Flemming, 1879).

Johann Friederich Miescher, a student of Hoppe Seyler (one of the pioneers of the then field "physiological chemistry), is a scientist born on August 13, 1844, in Basel, Switzerland who made the first revealing comment about DNA. During his work to describe the chemical composition of leukocytes, which he obtained from the pus on fresh surgical bandages, he showed that proteins and lipids were the main components of the cytoplasm, and he described their properties and attempted to classify them. Miescher had for the first time obtained crude DNA when he realised that a substance was precipitating upon the addition of acid and dissolved again when alkali was added (Dahm, 2005). However, based on histochemical facts, Miescher ascribed the material to the nuclei. He subsequently decided to closely study the cells' nuclei, which was not well known at the time. Miescher reported the then mysterious substance to Wilhem His in a letter on February 26, 1869, saying that "my experiments with low alkaline liquids, precipitates formed in the solutions after neutralization that could not be dissolved in water, acetic acid, highly diluted hydrochloric acid or in a salt solution, and therefore do not belong to any known type of protein". Thus, he realised that despite the substance having some properties of proteins, it was not a protein. Miescher referred to the enigmatic compound as "nuclein" due to its presence in the nuclei (Miescher-Rüsch, 1871).

Miescher's first protocol could not yield enough amount of the "nuclein" so he could not conduct further elementary analyses on the material. So, he then developed a second protocol that yielded enough amounts of the "nuclein". Miescher then proved that pepsin does not digest the "nuclein", he again realised that he could dissolve it with base and could reprecipitate it with excess acid. After conducting a solubility and digestibility test on the "nuclein" he realised that the "nuclein" varied from proteins in other ways too (Miescher-Rüsch, 1871). Miescher further conducted chemical tests and revealed that the "nuclein" contained various elements commonly found in organic molecules; carbon, oxygen, hydrogen and nitrogen, but, did not contain sulphur, unlike proteins. In addition, he reported that the "nuclein" contained a larger amount of phosphorus, which he previously reported to his parents in a letter he wrote on August 21, 1869. Without knowledge on the functioning of the "nuclein", Miescher wrote a letter on December 26, 1869, to Wilhem His with the speculation that further analyses to

determine the quantitative ratio of the “nuclein” and protein would promote a better distinction of pathological processes (Dahm, 2005).

In 1927, Gager and Blakeslee Miller also discovered that X-ray induced gene mutation (Gager and Blakeslee, 1927). A year later, Frederick Griffith also reported the genetic transformation of bacteria (Vasil, 2008). Hermann Joseph Muller, in 1946 discovered a genetic mutation in fruit flies induced by X-ray, for which he was awarded the Nobel Prize in physiology or medicine for his contribution. He reported that the higher the dose of X-ray and other ionizing radiation-exposed, the greater the number of mutations (Huang and Zhou, 2021; Muller, 1928; Muller, 1927). However, prior to that in 1944, Oswald Avery, Colin MacLeod and Maclyn McCarty had provided a piece of evidence demonstrating that our genetic material was DNA, and not protein as previously thought (Portin, 2014). Subsequently, in 1953, James Watson and Francis Crick after they researched the structure of DNA at the Cavendish Laboratory of Cambridge University in England, in an announcement “We have discovered the secret of life” published the structure of DNA (Huang and Zhou, 2021; Watson and Crick, 2007). Watson and Crick based their theories on the X-ray diffraction image of the DNA fibrils reported by Maurice Wilkins and Rosalind Franklin, in the team of Linus Pauling (Şenol, 2020).

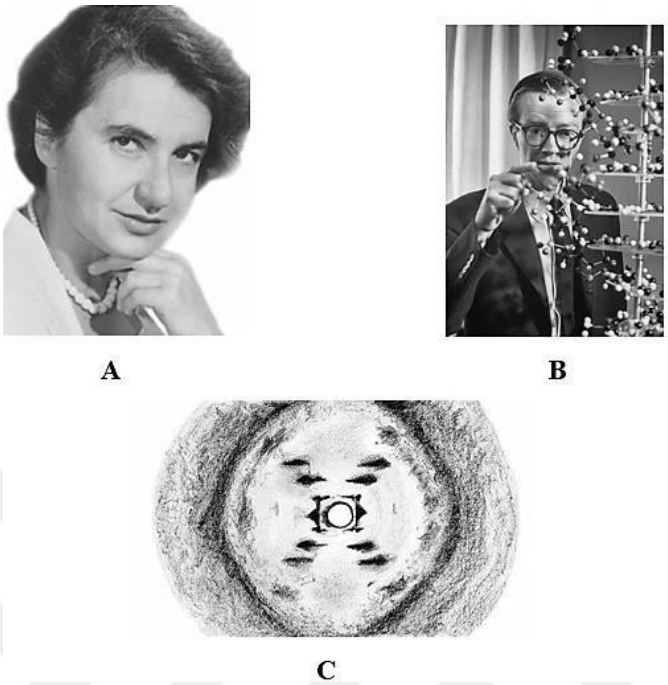

Figure 1.1 Rosanlind Franklin **(A)**, Maurice Wilkins **(B)** and their X-ray diffraction image of DNA **(C)** (Şenol, 2020).

Nowadays, the history behind the discovery of DNA is often told as though it began with these findings, the tremendous work conducted by Johann Friederich Miescher is sometimes neglected. Watson and Crick's determination of the three-dimensional structure of DNA is seen as the beginning of modern molecular biology and genetics studies.

1.3 Structure of DNA

Nucleotides are the fundamental building units of all nucleic acids. DNA is a complex polynucleotide, polyanionic biomolecule composed of three main constituents (Figure.1.2) namely; 1) a pentose sugar, 2'-deoxyribose sugar which occurs from the deoxygenation of ribose at the 2' carbon, hence, the name deoxyribonucleic acid 2) a purine or pyrimidine nucleotide base which is linked to the 1' carbon of the sugar via N-glycosidic bond. The purines are adenine (A) and guanine (G), the pyrimidines are cytosine (C) and thymine (T), and 3) a phosphate group, which infers the negative charge on nucleotides. It is attached to the 5' carbon or last carbon of the sugar via a phosphodiester bond.

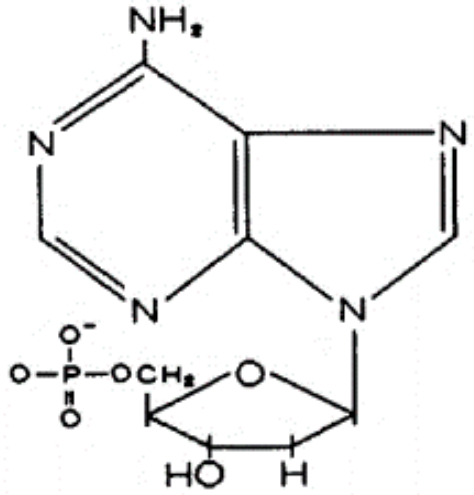

Figure 1.2 Fundamental building blocks of DNA, 2'-deoxyribose sugar, nucleotide base, and the phosphate group (Swiatek, 1994).

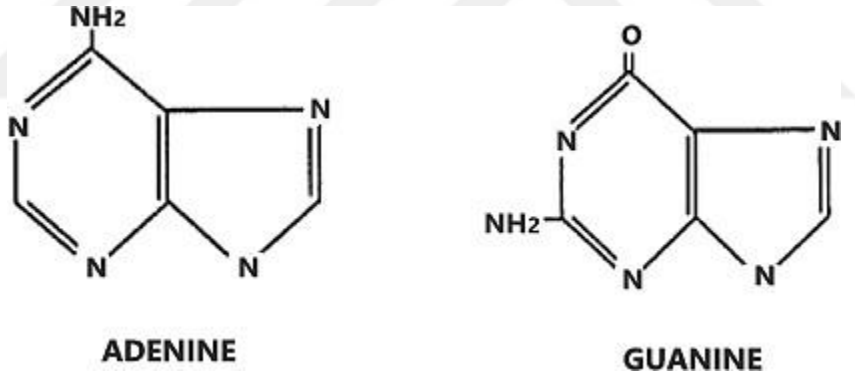

Figure 1.3 Purine Nitrogenous Bases (Swiatek, 1994).

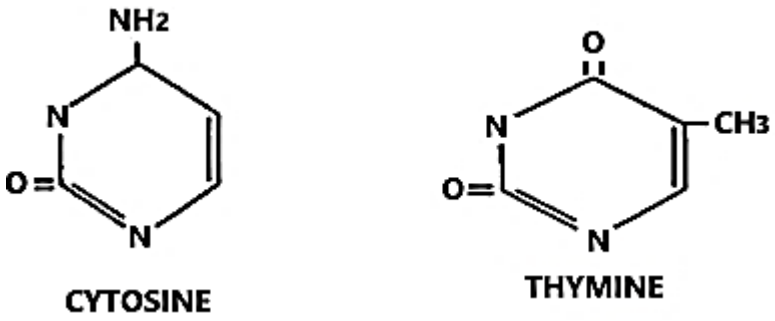

Figure 1.4 Pyrimidine Nitrogenous Bases (Swiatek, 1994).

The formation of a linear sequence by nucleotides is termed the primary structure of DNA. The primary DNA structure is formed via phosphodiester linkage which bonds the 5' of one nucleotide to the 3' of another (Figure. 1.5). When there is a complementary pairing between the nucleotide bases then the double-stranded DNA is formed. This complementary base pairing involves A pairing with T via two hydrogen bonds and C pairs with G via three hydrogen bonds (Figure.1.6). The dsDNA helix has its nucleotide bases at the hydrophobic centre and the sugar-phosphate backbone at the outside, according to the Watson and Crick model (Kanellis and dos Remedios, 2018). The nucleotide bases on both chains are stacked on each other 0.34 nm (3.4 Å) apart, the double helix has a diameter of 0.2 nm (20 Å) (Swiatek, 1994), and a length of 12 nm (Turel and Kljun, 2011). During the formation of the dsDNA helix (twisting), minor (small) and major (large) grooves are created. The size of these grooves and the exposed regions of DNA within these grooves dictates the type of bond and bond strength formed between DNA and the free molecules in its surroundings (Savreux-Lenglet et al., 2015).

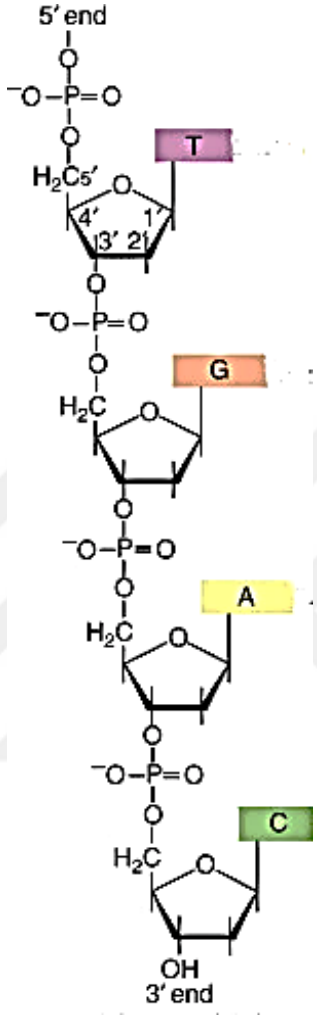

Figure 1.5 The primary structure of DNA.

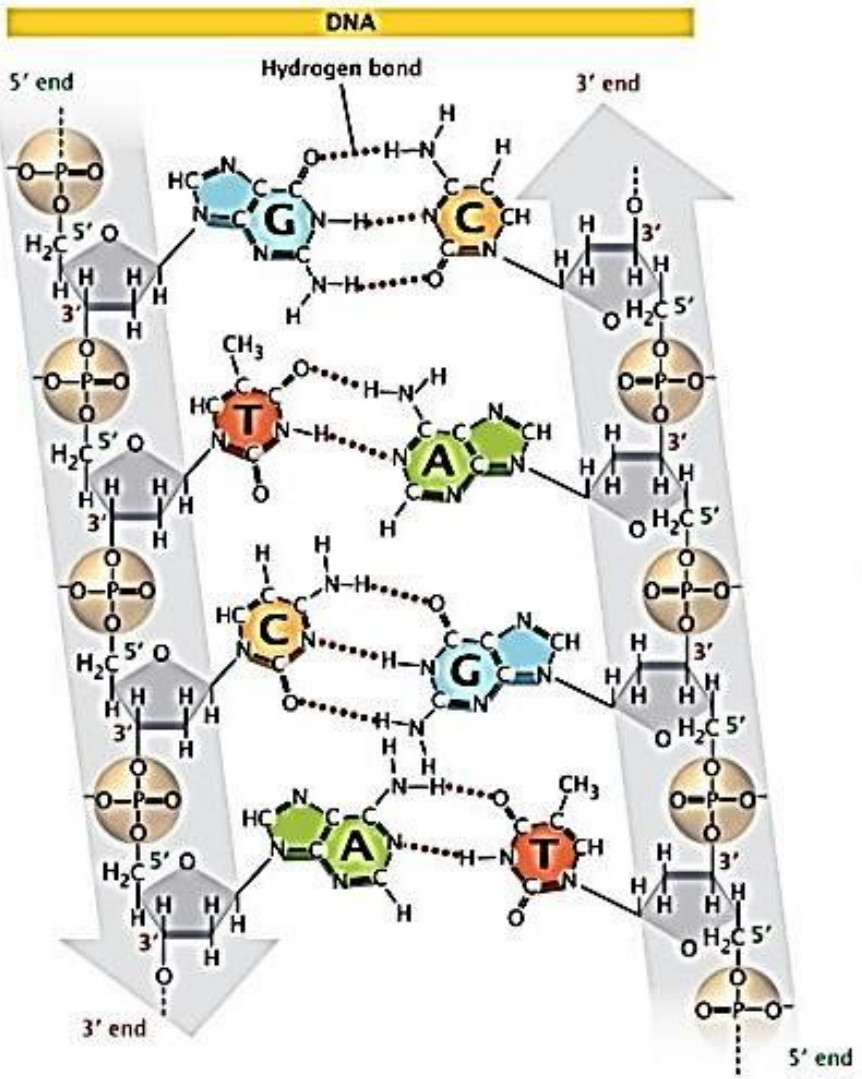

Figure 1.6 Double-helix DNA structure: Complementary base pairing of nitrogenous bases via hydrogen bond (Kanellis and dos Remedios, 2018).

1.4 Forms of DNA

Several structural forms of DNA helices have been identified and reported under various isolation, purification, and crystallization conditions. The first discovered forms of DNA were the A-form (alpha,α) and the B-form (beta, β) of DNA, the Watson and Crick's form of DNA (Swiatek, 1994). There is also the Z-helix form of DNA and collectively, these three forms are referred to as the biologically active forms of DNA (Kanellis and dos Remedios, 2018). Interestingly, there is also the C-form of DNA, which looks like the β-form of DNA with 9.33 nucleotide pairs per turn (Swiatek, 1994).

The β-form seems to be the naturally occurring form of DNA and the most common form of DNA. At normal physiological salt concentrations and neutral pH, the β-form forms a right-handed helical configuration with its sugar bases oriented at right angles to the structure (Kanellis and dos Remedios, 2018). β-form has a single turn double-helix length of ca. 12 nm and averagely, 10.4 π-stacked base pairs (Turel and Kljun, 2011). The canonical DNA has 10 base pairs per turn which are 3.4 Å stacked apart and a pitch rise per turn of 3.4 nm or 34 Å (Turel and Kljun, 2011). This form has a helix diameter of 20 Å, its minor groove is 4.8 Å wide, and the major groove is 10.5 Å wide (García-ramos et al., 2013). β-form of DNA can form several different structures based on certain conditions like; relative humidity, cations available, and base sequences. For instance, at a relative humidity of 92 % fibres of β-DNA are formed in the presence of alkali metal ions. Also, it can reverse to the α-form when relative humidity reduces to 75 % (Turel and Kljun, 2011). Generally, for the compaction of information into the human genome, uniformity to the Watson-Crick structure is required. Nevertheless, there are some exceptions to this uniformity, because unique secondary DNA structures can be formed by certain sequences. For instance; the repetitive pairing of guanines can give rise to G-quadruplex structures (Keniry, 2000) and also, the complementary pairing of cytosines can give rise to I-motif structures (Figure 1.7) (Hurley, 2002; Gehring et al., 1993).

A-DNA is also a right-handed helix composed of 11 base pairs per single turn. In this form of DNA, the nucleotide bases are 2.25 Å closer to each other and have a helix

diameter of 23 Å (García-ramos et al., 2013). α-DNA has a much wider, flatter, and shorter helical configuration. Exceptionally, its planes of base pairs are 20° tilted to the central/helix axis (Turel and Kljun, 2011). Hence, this form of DNA is more compacted with a hollow core than the B-DNA. The base pairs of A-DNA are not oriented at right angles to the helix axis, and its major and minor grooves have fewer structural similarities. Typically, A-DNA is formed from the dehydration of DNA.

The left-handed nature of the Z-form of DNA makes it exceptional from the A and B-forms (Figure 1.7). It is termed a Z-helix because of its zigzag shape. In this form, the conformational unit repeated is dinucleotide instead of a mononucleotide and the pattern of its backbone reflects the alternating guanine-cytosine (GC) conformation dinucleotide step. It has 12 base pairs per turn, a rise of 3.8 Å per residue, and an average helical twist angle per base pair of -30 °. The axis of this structure lies in the minor groove and as a result, the Z-DNA has a long and narrow appearance with its base pairs lying almost perpendicular to its axis (inclination of -6.2 °) (Lescrinier et al., 2003). Z-helix seems to be present along with the B-DNA under cellular conditions and it is probably crucial in the modulation expression (Swiatek,1994).

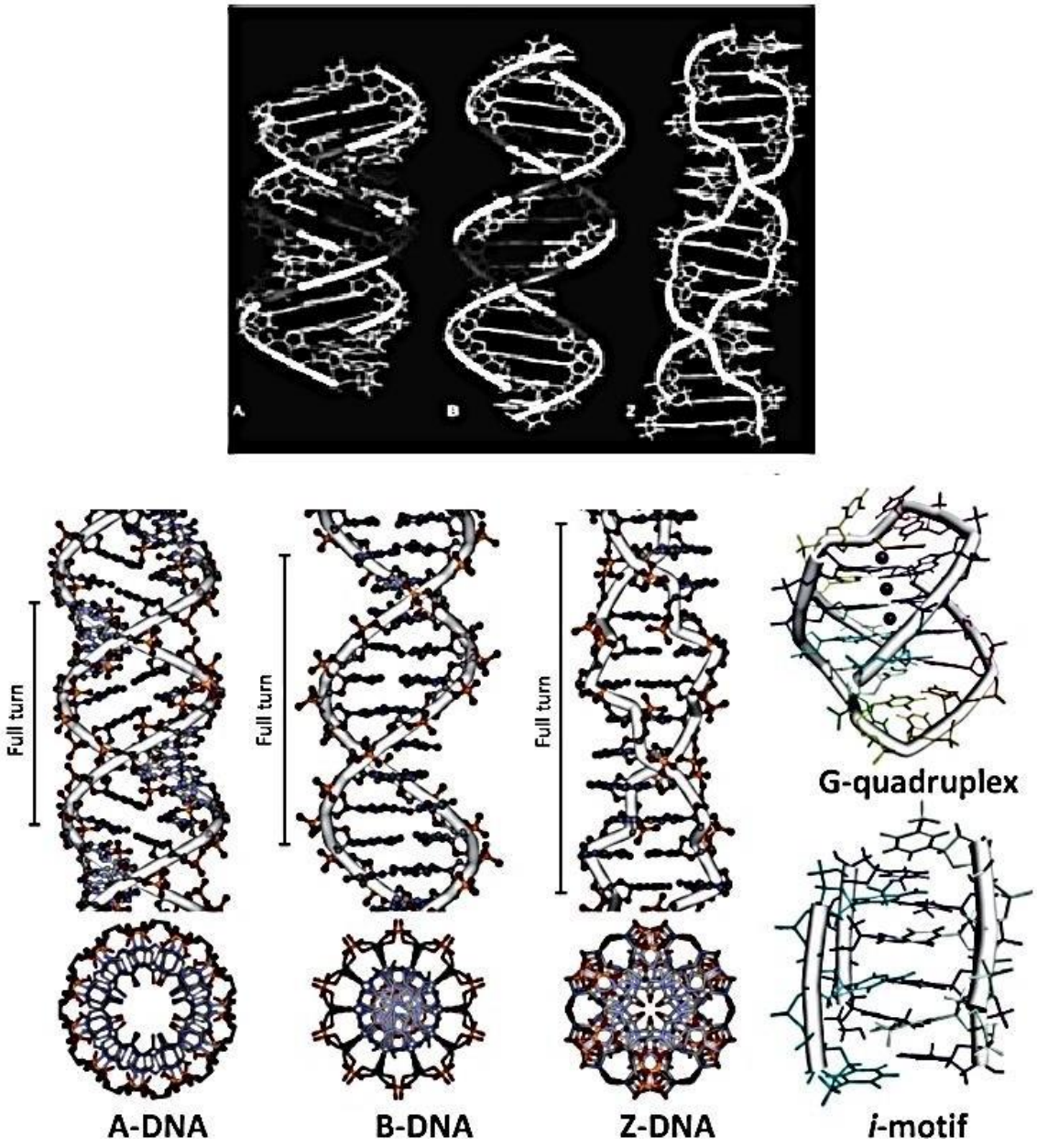

Figure 1.7 The biologically active DNA forms (A, B, and Z), G-quadruplex, and i-motif (Turel and Kljun, 2011; García-ramos et al., 2013).

1.5 DNA Binding Modes

Covalent Binding is a mode of DNA-binding occurring as a result of an interaction between a Lewis acid metal and nucleic acid Lewis bases, for instance, the electron-rich (nucleophilic) N7 residue of guanine. Covalent binding is generally kinetically controlled. However, the rate and metalation can be conveniently regulated by the initial reversible covalent binding (pre-association). Covalent binding is irreversible and enhances the activity of adduct species that prevent cell replication (Barone et al., 2013).

Figure 1.8 DNA-covalent binding mode (Pages et al., 2015).

Intercalation is a non-covalent DNA binding mode that involves the insertion of a DNA binding molecule or drug between neighbour pairs of bases (Barone et al., 2013). Metallo-intercalators are metal complexes having planar aromatic sections of the coordinating ligands. These ligands, orient parallel to the DNA base pairs and protrude away from the metal centre, thus, can easily π-stack in the DNA duplex. In other words, this mode of metal complex-DNA interaction involves specifically, the π-π interaction of the planar aromatic section of metal complexes with the stacked aromatic planes of the

nitrogen bases. The planar fragment size, shape, and type of substituents of the metal complex are essential as they help in distinguishing groove binders from intercalators (Lauria et al., 2014; Liu and Sadler, 2011; Zeglis et al., 2007). Intercalation interferes with the protein-DNA interaction due to the distortion it causes in the conformation of the DNA backbone. The latter may inhibit other DNA-associated functions such as; inhibiting the recognition and activities of enzymes like topoisomerases and polymerases, inhibiting DNA repair systems, and slowing down or even inhibiting transcription and translation processes (Barone et al., 2013). Intercalators function as frameshift mutagens. Even though basic, cationic or electrophilic functional groups are required for genotoxic activity, not all intercalators are genotoxic (Snyder and Hendry, 2005). Examples of reversible intercalators include the potent carcinogens ethidium bromine, proflavine (García et al., 2010), and benzopyrene (Metcalfe and Thomas, 2003).

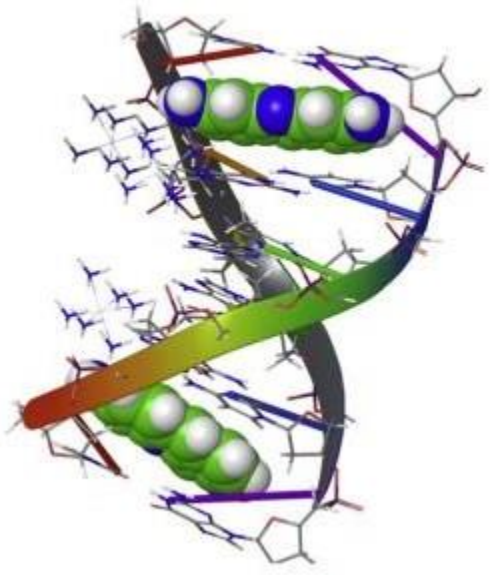

Figure 1.9 Illustration of intercalation binding mode (Barone et al., 2013).

With groove binding, the DNA grooves are negatively charged with high electrostatic potential, and this plays a vital role in groove binding. This DNA-binding mode is generally electrostatic in nature. It involves an interaction between a cationic metal complex and an anionic phosphate group. It may also occur as a result of the polar or cationic molecule forming a hydrogen bond with the oxygen and/or nitrogen atoms of the nucleotide bases or the backbone sugar (Lauria et al., 2014). This mode of interaction results in the neutralization of the negative charge of the phosphate backbone which tends to reduce the repulsive force between adjacent phosphate groups, thus, stabilizing the DNA structure. Covalent binding and intercalation are selective but electrostatic interaction is not, since it may occur at any phosphate site along the phosphate backbone (Barone et al., 2013).

Groove binding is either major or minor groove binding. These two types of groove binding differ from each other with respect to available interaction sites and size. Major groove consists of multiple interaction sites and has a relatively stronger interaction ability with the guest molecules (Kielkopf et al., 1998; Pabo and Sauer, 1984). It has a width of 11.6 Å and a depth of 8.5 Å (Neidle, 2001), which enhances the binding of bulky molecules to this site (Takeda et al., 1983). Contrary, minor grooves have lower binding sites and smaller sizes, a depth of 8.2 Å. Due to the smaller size of minor grooves, they may serve as the appropriate binding sites for smaller molecules like some antibiotics and anticancer drugs (Gao et al., 1992; Neidle, 2001).

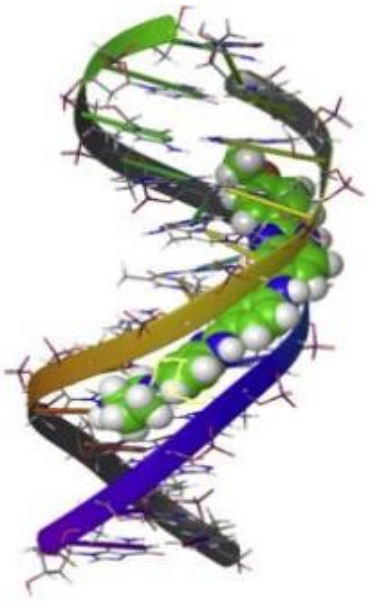

Figure 1.10 Groove mode of DNA-binding (Barone et al., 2013).

1.6 DNA-Metal/Metal Complex Interaction

Understanding the mechanism(s) of DNA-metal interaction and its biological effects is a subject of interest. The concept of agents-nucleic acids interaction is adopted in the study of genetic diseases and diseases caused by other agents like pathogens (Neidle and Thurston, 2005).

Regarding the size of the binding agent, a DNA interaction with a large binding agent induces alterations in the structural and conformational integrity of the DNA as well as causing the loss, addition or substitution of bases. These changes induced by the binding agent may eventually have an impact on the integrity of a genetic message, protein synthesis or may result in a change in enzyme activity (inactivation and/or specificity). When the mutation induced by the binding agent results in changes in the genetic information of a germinal cell, a progeny may inherit the mutation, and if the mutation causes a change in the genetic information of a somatic cell, then it may lead to an irreversible mutation that may incite tumour growth (Barone et al., 2013).

Free metals may interact with nucleic acids based on two distinct modes namely; 1) diffuse binding mode and 2) site binding mode. The diffuse binding mode involves the metal interacting with the nucleic acid in a delocalized manner. With this mode, the

positively charged metal retains its hydration layer and builds up around the nucleic acid, the interaction occurring through water molecules. This mode of binding is a long-range Coulomb interaction. The site binding mode, on the other hand, involves the metal coordinating to specific ligands on the nucleic acids. The coordination of the metal to ligands could be direct (inner spheres) or indirect, via water molecules (outer spheres). The nucleophilicity and steric effects of the coordination sites play a vital role in this binding mode (Hadjiliadis and Sletten, 2009). The nucleotide bases; guanine (G), adenine (A), cytosine (C), and thymine (T) have different affinities for metal ions. At normal physiological pH, metals prefer binding to the N7 guanine, N1 and/or N7 adenine, N3 cytosine, and O4 thymine sites of the nucleotide bases. Also, the type of metal ion influences the relationship between phosphate and nucleotide base. Hence, Eichhorn and Shin suggested that the order of relative metal affinity to the phosphate backbone of DNA is; $Mg^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+}$, considering metal-induced variation in thermal denaturation temperature (Tm) of DNA (Eichhorn and Shin, 1968). In contrast, it has also been reported that metals bind to nucleotide base residues in a sequence-dependent manner (Moldrheim et al., 1998). Owing to this, one may prefer designing metal complexes that can selectively bind to chosen DNA sequences. With regard to metal complexes, they interact with DNA by; 1) covalent binding and 2) non-covalent binding (intercalation and major or minor groove binding), explained above.

1.7 Techniques Used to Monitor DNA-Metal Complex Interaction

Despite the availability of a wide range of techniques used to study metal complex-DNA interactions, only X-ray and NMR can provide atomic-level details of the metal complex-DNA binding (Cachau and Podjarny, 2005; Andersen and Sletten, 2000; Han and Gao, 2000). Direct analysis of large and thermally labile biomolecules, the interaction of metal complexes with oligonucleotides and proteins can also be done adopting mass spectrometry, electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) methods (Beck et al., 2001; Mano and Goto, 2003). High-performance liquid chromatography (HPLC) is a technique also used to separate

and determine free metal ions and coordination compounds. It exhibits high speed and efficiency, and can be attached to several sensitive and selective detectors (Martins et al., 2007; Volckova et al., 2002 Constantinou-Kokotou et al., 2001). Under high electric field effect, based on charge, size, and frictional force, species can also be separated adopting the method, capillary electrophoresis (Sekhon, 2011; Righetti et al., 2002). Capillary electrophoresis upon being efficient and fast, provides valuable information on cooperativity, affinity, and selectivity (Araya et al., 2007; Hamdan et al., 1998). With different incubation protocols, gel electrophoresis can be used. These assays help in the verification or confirmation of intercalative binding (Barone et al., 2013; Zeman et al., 1998; Keck and Lippard, 1992), inhibition of topoisomerase (Webb and Ebeler, 2003), and DNA cleavage (Ruiz et al., 2010; Tan et al., 2009).

In determining metal complex-DNA binding mode, viscosity and sedimentation hydrodynamic are inevitably crucial methods. For instance; intercalation induces unwinding of the DNA double-helix due to the insertion of the intercalator or ligand into the base pairs. This insertion of the intercalator or ligand into the base pairs leads to an increased contour length, which subsequently induces an increase in viscosity and a decrease in the sedimentation coefficient. These changes are absent in groove-binding (Barone et al., 2013; Cohen and Eisenberg, 1969). Linear dichroism (LD) enhances the determination of the metal-complex orientation along the DNA long axis. Small metal complexes in solution do not orient and hence, do not give LD signals; thus, the presence of LD is proof that the metal complex is bound to the DNA. Intercalators that closely stack in base pairs give LD signals similar to base pairs. Groove binders, on the other hand, provide signals that are opposite to that of intercalators (Barone et al., 2013; Kurucsev and Kubista, 1992). Circular dichroism (CD) is a method that also provides insights into the DNA-metal complex binding. Normally, non-chiral binding agents do not give signals, but when CD is induced a signal is produced. Hence, an induced CD signal in the absorption region of a non-chiral binding agent proves DNA binding (Barone et al., 2013; Pérez-Rentero et al., 2015).

Ultraviolet (UV) VIS absorption and fluorescent spectrometry are also methods used due to their good sensitivity, simplicity, reproducibility, and versatility. The absorption and fluorescent spectra of metal complexes or binding agents change upon

binding to nucleic acids, shifting the maxima. During the interaction of these agents with nucleic acids, fluorescence enhancement or quenching can be observed (Busto et al., 2011).

1.8 Copper DNA Binding Activity

The interaction existing between metal complexes and DNA has chemical and biological properties on the molecular system. Metal-based drugs, metallodrugs have their cytotoxic activities associated with their DNA binding properties. For this reason, there is a current concern on synthesizing metal complexes as potential DNA binders. Generally, the interaction modes of metal complexes with DNA have been categorized into three main groups namely; (1) covalent binding DNA-alkylators, (2) major or minor grooves binding and (3) intercalation. The latter two are sometimes collectively referred to as non-covalent binding mode. Mixed modes of binding also exist as some intercalators like anthracyclines interact by electrostatic attraction or hydrogen bonding with atoms exposed in grooves (Lauria et al., 2014).

A study conducted by Lauria et al. (2014) to investigate the structural details of DNA binding by Cu (II) Schiff base complex with calf thymus DNA (ct-DNA) reported that Cu (II) exhibited the intercalative mode of interaction with the DNA, with an intrinsic binding constant (*K*b) of 2.9 x 10^6. In an experiment to ascertain the cleavage and DNA-binding modes of three macrocyclic Cu (II) complexes; $\{CuL^1\}$ Cl_2, $\{CuL^2\}$ Cl_2 and $\{CuL^3\}$ Cl_2 with calf thymus DNA, it was observed that $(CuL^1)^{2+}$ showed a partial intercalation binding mode through the pyridine ring into the DNA base pairs. $\{CuL^2\}^{2+}$ bind to the DNA via hydrogen bonding and hydrophobic interaction while $\{CuL^3\}^{2+}$ bind to the DNA via weaker hydrogen bonding. It was also noticed that all the three Cu (II) complexes cleaved DNA in the presence of 2-mercaptoethanol and hydrogen peroxide (H_2O_2) (Liu et al., 2002). Furthermore, spectrophotometric and viscometry results suggested that Cu (II) complex binds to DNA via intercalation. An evaluation of the trend of the *K*b values of L1-L4 complexes of Cu suggested that the increased planarity of the L1-L4 Cu complexes appeared to have enhanced their intercalation ability (Barone et al., 2013). In addition, novel Cu (II) complexes that were synthesized by interacting Cu (II) with the non-steroidal anti-inflammatory drug

meclofenamate in the presence or absence of nitrogen-donor co-ligand exhibited an intercalation mode of binding to calf thymus DNA. The binding strength of these complexes was further investigated by conducting a competitive study with ethidium bromide (EB), and it was observed that the complexes were able to displace the EB off the EB-DNA adduct, which confirms the intercalative binding mode (Barmpa et al., 2021).

In a study conducted to investigate the interaction of the two Cu (II) drugs; CuL(NO3) and CuL'(NO3) (pyridine-2-carbaldehyde thiosemicarbazone and pyridine-2-carbaldehyde 4N-methylthiosemicarbazone) in water named [CuL] and [CuL']) with [poly(dA–dT)]2, [poly(dG–dC)]2, and with ct-DNA in an aqueous solution of pH 6.0, it was reported that the drugs interacted with [poly(dA–dT)]2 as groove binders with a site size of n=6-7, and as external binders with [poly(dG–dC)]2 and/or CT-DNA. The study also reported that the compounds exhibited DNA cleavage via the generation of ROS, by the reoxidation of Cu (I) to Cu (II) in the presence of thiols. However, no DNA cleavage was observed in the absence of thiols (Ruiz et al., 2010).

Copper sulphate ($CuSO_4$) is the known common Cu salt. Isolated rat hepatocytes when incubated with $CuSO_4$, chromosomal aberrations were observed. It is considered to be genotoxic and for this reason, its genotoxicity has been evaluated but, there are discrepancies surrounding its genotoxicity (Saleha Banu et al., 2004; Sina et al., 1983). Eleven $CuCl_2$ compounds synthesized with ligands interacted with the DNA of tumour cells via intercalation. The spectrophotometric results revealed that the complexes had a strong affinity for the DNA as intercalators and also, they induced conformational changes in the DNA. Molecular docking studies, again revealed that the compounds interact with DNA by π-π stacking, hydrophobic interactions, Van Der Waals forces, and hydrogen bonding (Li et al., 2021). An *in-vitro* investigation of the DNA interaction activity of $CuCl_2$ complex from amidino-O-methylurea ligand reported that the complex at low ({complex}/{DNA}) ratios manifested a non-intercalation mode of binding and manifested intercalation binding mode at high ({complex}/{DNA}) ratios. Furthermore, the nuclease activities of the complexes were evaluated by gel-electrophoresis and atomic-force microscopy. The results showed that the complexes can cleave plasmid pBR322 DNA, probably by oxidative DNA damage (Meenongwa et al., 2016).

1.9 Pharmaceutical Applications of Metal Complexes

Undisputedly, metal complexes play tremendous roles in the pharmaceutical industry as anti-cancer agents, anti-diabetic agents, anti-inflammatory agents, anti-parasitic agents, and anti-microbial agents. Metal complexes such as platinum (II) complexes since time immemorial are used in chemotherapy, and among this group of metal complexes, cisplatin has showed high chemotherapeutic potency (Jamieson and Lippard, 1999; Rafique et al., 2010). Cisplatin, through the formation of cisplatin-DNA adducts at the guanine portions of DNA as those portions are electron-rich, induces distortion, and inhibits DNA replication (Lee et al., 2002). The formation of cisplatin-DNA adducts is vital as it serves as a binding site for the high-mobility group protein (HMG-protein). The binding of the HMG-protein to the cisplatin-DNA adducts has been reported to be an essential characteristic that contributes to the anticancer effects of cisplatin (He et al., 2000). In spite of its efficacy, just as other drugs, the usage and efficacy of cisplatin is sometimes limited by drug resistance and resistance to it has been reported in about 20 % of patients with metastatic cancer (Piulats et al., 2009). Owing to the cytotoxicity of cisplatin to normal cells, coupled with the resistance to it, efforts are strenuously being made to enhance its efficacy by improving its specificity to tumour cells through conjugation with molecules like porphyrins (Lottner et al., 2002).

Due to the shortfalls of cisplatin, other platinum complexes having their DNA binding modes different from that of cisplatin have been suggested to be crucial in the treatment of cancer as they exhibit anticancer activity against cisplatin-resistant cancer cells. For instance, trans-diaminedichloroplatium (II) (DDP) has been reported to have anticancer activity against ovarian cancer cell lines that are resistant to cisplatin (Najajreh et al., 2002). Hence, the clinical usage of oxaliplatin, carboplatin, and nedaplatin (Rafique et al., 2010).

Aside from platinum complexes, other metal complexes including Titanium complexes are used in the treatment of cancer (Chen et al., 2009). For instance, Titanocene dichloride, which is a Titanium-based known anticancer drug, is used in the treatment of breast and gastrointestinal carcinomas (Rafique et al., 2010). In a study conducted by Marcon et al. (2002) to investigate the DNA binding activities and

cytotoxic effects of gold (III) complexes synthesized with bipyridyl ligands on tumour cell lines reported that the gold (III) complexes manifested cytotoxic effects on the tumour cell lines. Also, when the cytotoxic activities of some gold (III) complexes were studied *in vitro* through the sulforhodamine B assay on human ovarian cell lines, it was reported that they showed relevant cytotoxic properties with IC_{50} values within the range of 0.2-10 µM (Messori et al., 2000). In addition, Mn (III)-Salen incites apoptosis in human cancer cells. A study conducted, Mn (III)-Salen instigated the activation of caspase-3/7 and the release of cytochrome-c from the mitochondrial into the cytosol. Implying that Mn (III)-Salen exhibited its anticarcinogenic effect via the mitochondrial pathway. Mn (III)-Salen derivatives induced apoptosis in breast cancer cell lines at IC_{50} within the range of 11-40 µM, thus, they can be considered as novel anticancer agents (Ansari et al., 2009).

Apart from the anti-carcinogenic properties of metal complexes, they also possess anti-diabetic effects. Currently, the treatment of diabetes also depends on chemotherapies intended to reduce hyperglycemia in patients, together with exercise and diet control. However, the efficacies of these therapies are limited, tolerance to these therapies are limited and also, they have significant mechanism-based negative effects. Thus, the need for alternative approaches was urgent. In the quest for alternative treatment approaches for diabetes, studies and experiments were carried out. During the struggle for alternative approaches, it was found that metals and their complexes have anti-diabetic effects (Sakurai et al., 2008). The administration of vanadium and zinc as inorganic salts has been reported to have resulted in a control of blood plasma glucose levels (Rafique et al., 2010). Vanadium when in the form of vanadate (with the oxidation state 5^+) and in the form of vanadyl (with the oxidation state 4^+) can trigger the uptake of glucose by cells and glycogen synthesis. In other words, in those states, vanadium can mimic the activities of insulin (Chen et al., 2009). Also, the supplementation of chromium to diabetic patients and non-diabetic individuals showed that chromium significantly improved glycemia among the diabetic patients but did not show any effect on the metabolism of glucose and lipid in the non-diabetic individuals (Balk et al., 2007). Patients having type 1 and type 2 diabetes often have lower blood levels of zinc (Ripa and Ripa, 1995). A higher intake of zinc probably lowers the risk of developing

type 2 diabetes in women (Sun et al., 2009). This implies that zinc has an insulin-mimetic activity and antidiabetic activity. Nonetheless, the antidiabetic properties of zinc complexes have not been fully exploited (Sakurai et al., 2008).

Metal complexes are also used in the treatment of arthritis, as anti-inflammatory agents. For instance, the gold complexes; sodium aurothiomalate, sodium aurothioglucose, and sodium aurothiopropanol are used clinically to treat severe rheumatoid arthritis. Inflammatory is often accompanied by angiogenesis. Hence, the use of anti-angiogenesis agents during the treatment of inflammatory cases helps in faster amelioration. This can be achieved using a heparin derivative conjugated with silver or gold nanoparticles as an anti-angiogenesis agent (Rafique et al., 2010). Oxidative injury, which occurs as a result of oxidative stress can lead to chronic inflammation. Conversely, mitigating the occurrence of oxidative injury can help prevent or reduce cases of inflammation. Ruthenium (III)-quercetin complex has demonstrated this ability to reduce oxidative stress and oxidative injury back to normal levels in both the brain and testis (Refat et al., 2021). Lipopolysaccharide induces the secretion of the pro-inflammatory cytokine, tumour necrosis factor-alpha (TNF-α) and this inducing effect of lipopolysaccharide on the secretion of TNF-α has been reported to have been inhibited by ferracyclic ion (II) complexes bearing two carbonyl ligands. In other words, the complexes exhibited an anti-inflammatory potency by inhibiting the effect of lipopolysaccharide (Wright et al., 2020).

In addition, metal-based compounds are used to treat vital tropical diseases such as Chagas disease, leishmaniasis, and malaria (Navarro and Visbal, 2021). In the treatment of leishmaniasis and trypanosomiasis, bismuth and arsenic have been recognized to have potency against these conditions, respectively (Bagchi et al., 2015). Also, Cu (II) and gold (I) clotrimazole have manifested significantly higher inhibition on the growth of *Trypanosoma cruzi* (the cause of Chagas disease) (Navarro et al., 2001). Metals do also have anti-microbial properties and typically, the majority of the metals having anti-microbial activities are those of the d-block (V, Ti, Cr, Co, Ni, Cu, Zn, Tb, W, Ag, Cd, Au, and Hg). Some metals and metalloids belonging to groups 13-16 of the periodic table also have anti-microbial activities (Al, Ga, Ge, As, Se, Sn, Sb, Te, Pb, and Bi) (Shakeri et al., 2019; Turner, 2017). Silver is used widely as an anti-infective agent.

For instance, silver sulfadiazine is used as both an anti-microbial agent and as an anti-fungal agent to mitigate microbial infections during cases of severe burns. Furthermore, the highly fluorinated silver complex (silver(I) tris(pyrazolyl)borates) has good anti-microbial efficacy against *Staphylococcus aureus* (Dias et al., 2006). In some countries, to prevent ophthalmia neonatorum in newborns, 1 % silver nitrate ($AgnO_3$) solution is used to wash the eyes of newborns(Chen et al., 2009). Moreso, Co (II), Ni (II), Zn (II), and Cu (II) complexes have a good anti-microbial activity (Palaniammal and Vedanayaki, 2021).

1.10 Study Objective

This thesis aimed to investigate the interaction of genomic DNA (calf thymus DNA) with some copper compounds ($CuSO_4$, $CuCO_3$ and $CuCl_2$) and their possible cleavage activities, *in vitro*. The studies carried out in this context purposed to provide information and add to the existing literature on the possible therapeutic potentials of copper compounds, precisely, $CuSO_4$, $CuCO_3$ and $CuCl_2$.

2. MATERIALS and METHODS

UV-absorbance spectrophotometry, fluorescence spectrophotometry, and agarose gel electrophoresis analyses were adopted in this study. They were performed in the Molecular Biochemistry Laboratory of Kırıkkale University, Faculty of Veterinary Medicine, Department of Biochemistry. The fluorescence spectrophotometry technique was performed in Konya Food and Agriculture University, KIT-ARGEM Research Center.

2.1. Thymus Tissue

The isolated DNA used in this study was isolated from bovine thymus tissue and this is because, the bovine thymus tissue is a rich source of nucleic acids, thus, enhancing adequate isolation of DNA. Ethical approval was obtained from the Kırıkkale University Animal Experiments Local Ethics Committee before the bovine thymus tissues were collected (55762/02.11.2021). The bovine thymus tissue was obtained from Hasandede Municipality Slaughterhouse and in a cold chain, was brought to the Molecular Biochemistry Laboratory of Kırıkkale University, Faculty of Veterinary Medicine, Department of Biochemistry. Before usage for DNA isolation, the tissue was stored at -80 °C.

2.2. Sterilization Process

With an autoclave device, the automatic pipette tips and Eppendorf tubes that were used in this study were sterilized at 120 °C for 20 minutes (Nüve steamArt OT, Turkey). On the other hand, all of the glassware used in this study was chemically disinfected with 10 % nitric acid (Tekkim®, Turkey) and rinsed 3 times with distilled water. Subsequently, they were dried in an oven (Memmert UN-110, Germany) at 120 °C for 60 minutes (1 hour).

2.3. Chemicals Used

Table 2.1. List of chemicals with their codes and countries of origin

Name of Chemical	Code, Company and Country of Origin
Copper (II) carbonate ($CuCO_3$)	61167, Merck, Germany
Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$)	AM0686890527, Merck, Germany
Copper (II) chloride ($CuCl_2$)	S6484147219, Merck, Germany
Agarose	D00103, Pronadisa, Spain
Boric acid (H_3BO_3)	K30746365, Merck, Germany
Tris ($H_2NC(CH_2OH)_3$)	K31626787, Merck, Germany
EDTA	3A27866, Bioshop, Canada
6x DNA loading dye	00158610, Thermo Scientific, USA
6x EZ-vision dye	1576C339, VWR Life Science Biotechnology, USA
SafeView nucleic acid dye	G108, ABM, Canada

2.4. Solutions and Preparation of Solutions

2.4.1. Preparation of Copper (II) Carbonate, Copper (II) Sulphate pentahydrate, and Copper (II) Chloride Solutions

The aqueous solutions of the copper compounds ($CuCO_3$, $CuCl_2$, $CuSO_4.5H_2O$) used in the study were prepared by dissolving them in ultrapure water at the concentrations decided from the preliminary experiment. Solutions of 1000, 500, 250, 125, and 62.5 µM concentrations of all three compounds were prepared and stored in black bottles.

2.4.2. Preparation of Tris-Boric acid-EDTA Buffer Solution (TBE)

10x stock TBE buffer solution was prepared for use in the agarose gel electrophoresis technique. For this purpose, 108 g of tris, 55 g of boric acid, and 9.3 g of EDTA were weighed with a precision balance (Radwag PS 510-R1, Turkey) and dissolved in a flask with 800 ml of sterilized ultrapure water. After the thawing process, the stock solution, which was made up to 1000 ml with sterilized ultrapure water, was stored at room temperature, and away from light in a cabinet. The stock solution was diluted with ultrapure water and used at 1x concentration for the electrophoresis studies. The 1x concentration was prepared by mixing 100 ml of the 10x stock solution with 900 ml of ultrapure water. The buffer solution was then used to dissolve the agarose and to enhance conductivity in the electrophoresis tank.

2.5. DNA Isolation

Wizard® Genomic DNA Purification Kit (A1120, Promega, USA) was used for the DNA isolation

The kit's protocol was implemented using the following materials;

- Cell lysis solution (A1120, Promega, USA)
- DNA rehydration solution (A1120, Promega, USA)
- Core lysis solution (A1120, Promega, USA)
- Protein precipitation solution (A1120, Promega, USA)
- RNase solution (A1120, Promega, USA)

- 2-propanol (K29459195, Merck®, Germany)
- 70 % Ethanol (SZBC1160V, Sigma Aldrich®, USA)

DNA isolation from the thymus tissue was performed with strict adherence to the kit's protocol, and the following steps were carried out;

1- 20 mg of bovine thymus tissue was homogenized with a homogenizer (Stuart-SHM1 Homogeniser, UK) for 20 seconds in a 3 ml sterile tube upon adding 600 µl core lysis solution at +4 °C.
2- Following homogenization, the homogenized tissue-core lysis solution mixture was taken into a 1.5 ml sterile Eppendorf tube and 3 µl of RNase solution was added to it. Using a block incubator (Benchmark BSH1001-E, USA), it was then incubated at 37 °C for 30 minutes.
3- After incubation, the mixture was allowed to stand for 5 minutes at room temperature. 200 µl of protein precipitation solution was added and mixed for 30 seconds with a vortex device (Velp Scientifica-ZX3, Italy), and then kept on ice for 5 minutes.
4- The resultant mixture was centrifuged at 15000 xg for 4 minutes (ISOLAB Laborgerate, 603.02.001, Germany).
5- After centrifugation, the supernatant was transferred to another Eppendorf tube. 600 µl of 2-propanol at room temperature was then added to it and mixed gently turning it upside down.
6- The supernatant-2-propanol mixture was centrifuged for 1 minute at 15000 xg.
7- The following centrifugation, the supernatant was discarded and 600 µl of 70 % ethanol at room temperature was added to the residue at the bottom of the Eppendorf tube.
8- The residue-70 % ethanol mixture was centrifuged at 15000 xg for 1 minute.
9- After centrifugation, the supernatant was discarded. To evaporate any possible remaining ethanol, the white residue at the bottom of the Eppendorf tube containing the DNA was allowed to air dry.
10- After allowing the white residue to air dry, 100 µl of DNA rehydration solution was added and incubated at 65 °C for 60 minutes in an oven (Memmert UN-110, Germany). Eppendorf tubes were gently mixed at regular intervals during the incubation period and this climaxed the process of DNA isolation.

The purity and concentration of the DNA isolated from the bovine thymus tissue with the Wizard® genomic DNA purification kit was determined with a micro-drop spectrophotometer (Multiskan GO, Thermo Scientific, USA). Measurements were done with a micro-drop plate (Figure 2.1) using 3 µl of the isolated DNA each. The 260/280 absorbance values and concentrations (ng/µl) of the samples were determined. After providing relevant data, stock DNAs were stored at -80 °C.

Figure 2.1 Analysis of DNA concentration and purity by a micro-drop spectrophotometer.

2.6. UV-Absorbance Spectrophotometry Technique

UV-absorbance spectrophotometry is one of the techniques used to investigate or study DNA-molecules interaction. It was conducted using a Multiskan GO UV-absorbance spectrophotometer. After the device was calibrated with nuclease-free water, with a Nano plate, the isolated bovine thymus DNAs were measured with the various copper compounds that were prepared in different concentrations, separately. The

copper compounds were mixed with the bovine thymus DNAs to determine their interaction, in 0.2 ml sterile Eppendorf tubes. The mixing was done at a ratio of 1:1 and incubated at 37 ℃ for 60 minutes. After that, the absorbance of the DNA + copper compounds were measured and relevant graphs were generated.

2.7. Fluorescence Spectrophotometry Technique

To confirm the binding capacity and interaction of the copper compounds with the ct-DNA, fluorescence spectroscopy was conducted. For this purpose, the various concentrations (62.5 µM, 125 µM, 250 µM, 500 µM, 1000 µM) of the copper compounds; $CuSO_4.5H_2O$, $CuCl_2$, and $CuCO_3$ were mixed with the ct-DNA in a 1:1 ratio. 5 µl of each concentration of the copper compounds was mixed with 5 µl DNA (1 µg/µl), and incubated at 37 °C for 1 h in a water bath. Following incubation, 180 µl DNase/RNase free ultrapure water was added to each well of the 96-well flat-bottom black plate (Thermo Nunc 237105, Denmark) (Figure.2.2) and then, the 10 µl copper compound-DNA mixture was added. Finally, 10 µl DNA dye (SafeView Classic, abm®, Canada, G108) diluted in a 1:15 ratio with sterile ultrapure water was added in duplicate to the wells. All concentrations of the copper compounds (10 µl each) were mixed with 10 µl of the diluted (1:15) DNA dye and without DNA dye (only copper compounds), and were measured as the positive control. Also, only DNA dye, DNA with DNA dye and, DNA without DNA dye were measured as the negative control. Before measurements, all the contents in each well of the 96-well black plate were well mixed with a multichannel pipette. The fluorescence intensities (as RFU (Relative Fluorescence Units)) of the 96-well plate were measured at 290 nm excitation value (λex) and within an emission range (λem range) of 400-700 nm in a multimode microplate reader (Synergy H1M, BioTek, USA) using Gen 5 (version 3.00.19) software (Figure.2.3).

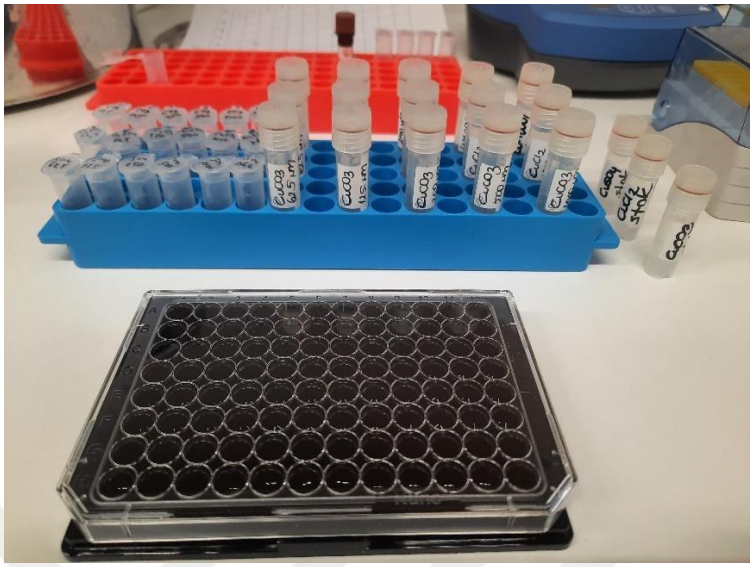

Figure 2.2 Image of 96-well flat bottom black plate.

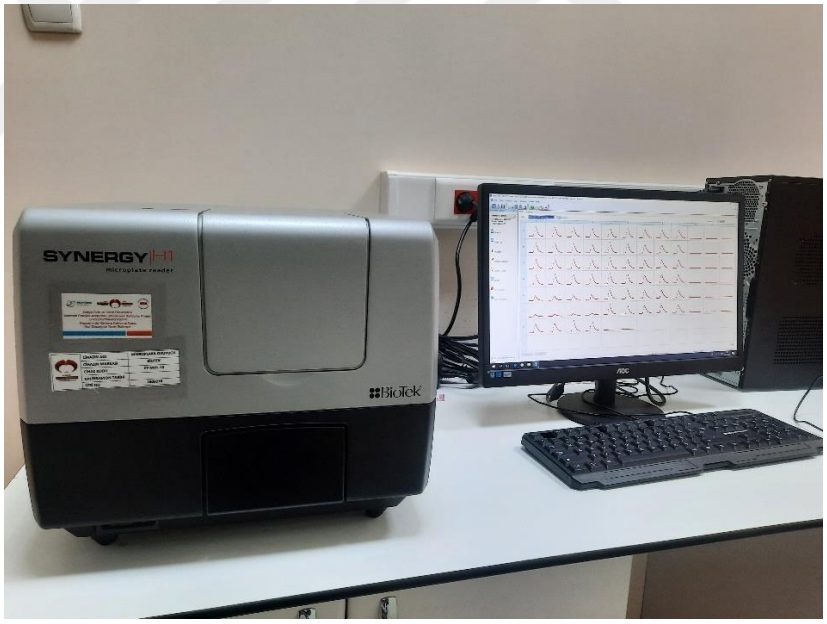

Figure 2.3 Measurement of fluorescence intensities.

2.8. Agarose Gel Electrophoresis Technique

In this study, the agarose gel electrophoresis studies were conducted with a horizontal electrophoresis system (nanoPAC-300, Cleaver Scientific, UK). Preliminary studies were done under electrophoresis conditions to optimize the gel density, electric

current, and running time values. Eventually, a gel density of 1.5 %, an electric current of 100 Volts, and a running time of 1.5 hours was used in the study.

2.8.1 Preparation of Samples

Samples were prepared by combining the genomic DNAs with the various concentrations of the copper compounds in a 0.2 ml sterile Eppendorf tube, at a ratio of 1:1. 5 µl of DNA and 5 µl of each copper compound were mixed in tubes by repetitive pipetting. The resultant mixtures were then incubated at 37 ℃ for 60 minutes.

2.8.2 Preparation of The Agarose Gel

In preparing the agarose gel, a commercially procured agarose (Pronadisa, Spain) was used. 1.5 g of agarose was mixed with 100 ml of 1x TBE buffer solution in a flask. It was then placed in a microwave oven (Vestel MD-20MB, Turkey) for 2 minutes, for it to dissolve under the application of high heat. In order to reduce the temperature to 40 ℃, the gel solution was shaken and allowed to cool under the tap. After that, the gel was poured into a gel tray to solidify and the combs were placed at their appropriate positions. It was then left at room temperature for 30 minutes. When the gel was ready, the combs were removed and placed together with the gel tray into an electrophoresis tank (nanoPAC-300, Cleaver Scientific, UK).

2.8.3. Loading of Samples

After the process of incubation, 10 µl of the samples to be electrophoresed DNA + copper compounds were loaded into the gel wells after they were mixed with 2 µl of DNA loading dye and 2 µl of Ez-vision dye.

2.8.4. Process of Electrophoresis and DNA Bands Imaging

Following the process of loading, the electrophoresis process was carried out at 100 volts for 1.5 hours. UV transilluminator (WiseUV WUV-L2O, Germany) was used to visualize the bands that were formed in the gels. The gel photographs were recorded using an imaging system (Gel Doc^{TM} EZ, Bio-Rad, Turkey) and the DNA band intensities (Int) were evaluated with a software (Image lab 6.0, Bio-Rad, Turkey).

3. RESULTS

3.1. DNA Isolation

The concentration of the ct-DNA isolated and used within the scope of this study was 1.84 at Å 260/280. The purity and concentration value of the DNA obtained at the end of the DNA isolation process was found to be appropriate for the conduction of UV-absorbance spectrophotometry, fluorescence spectrophotometry and agarose gel electrophoresis.

3.2. UV-absorbance Spectrophotometry

Before the measurement, a 1:1 combination of ct-DNA and the five different concentrations of the copper compounds was prepared and incubated at 37 ℃ for 1 hour. From the data obtained from the UV-absorbance spectrophotometry, as expected, the copper compounds did not give absorbance peaks at 260 nm (Figure. 3.2, 3.3, and 3.4). Also, it was observed that in addition to the control, the combinations of ct-DNA with the various copper compounds' concentrations gave wave peaks at 260 nm. According to the graphs, it can be inferenced that as the concentrations of the copper compounds increase, the wave peaks of the ct-DNA also increase. In other words, an increase in the concentration of the copper compounds resulted in hyperchromism. $CuCl_2$ had the strongest interaction with DNA at 1000 μM within the absorbance range of 220-320 nm (figure. 3.5). Within the same absorbance range, $CuSO_4$ and $CuCO_3$ also produced their greatest hyperchromic effect on DNA at 1000 μM (figure. 3.6 and 3.7, respectively).

Table 3.1. UV-absorbance results of copper compounds with ct-DNA

		230 nm	260 nm	280 nm	320 nm
$CuCl_2$	1000 µM	0.608	0.883	0.557	0.112
	500 µM	0.552	0.829	0.499	0.100
	250 µM	0.510	0.753	0.459	0.094
	125 µM	0.487	0.727	0.434	0.093
	62,5 µM	0.464	0.697	0.414	0.087
$CuSO_4$	1000 µM	0.634	0.864	0.577	0.125
	500 µM	0.521	0.770	0.484	0.096
	250 µM	0.539	0.815	0.495	0.098
	125 µM	0.477	0.708	0.423	0.089
	62,5 µM	0.376	0.548	0.338	0.075
$CuCO_3$	1000 µM	0.623	0.883	0.583	0.122
	500 µM	0.537	0.795	0.493	0.098
	250 µM	0.485	0.709	0.433	0.097
	125 µM	0.480	0.704	0.426	0.094
	62,5 µM	0.442	0.639	0.392	0.091
Control DNA		0.914	1.475	0.876	0.169

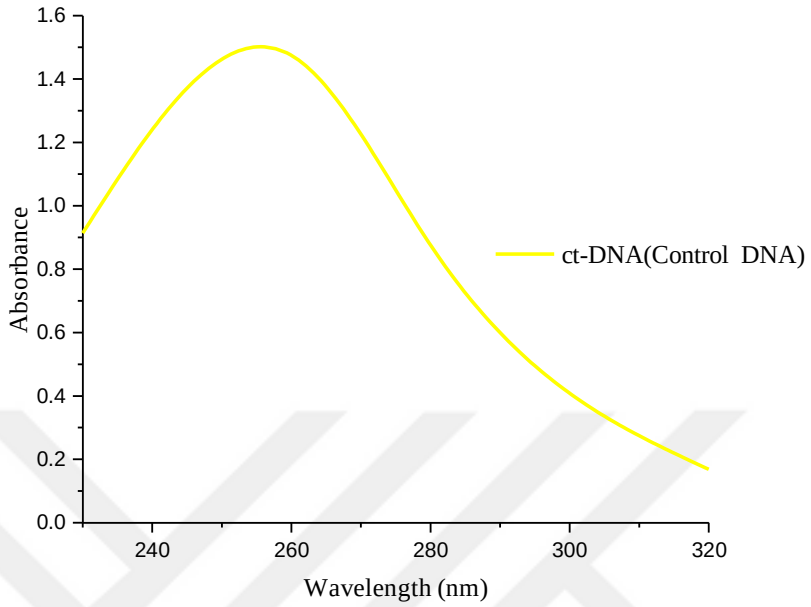

Figure 3.1 UV-absorbance graph of ct-DNA (Control DNA).

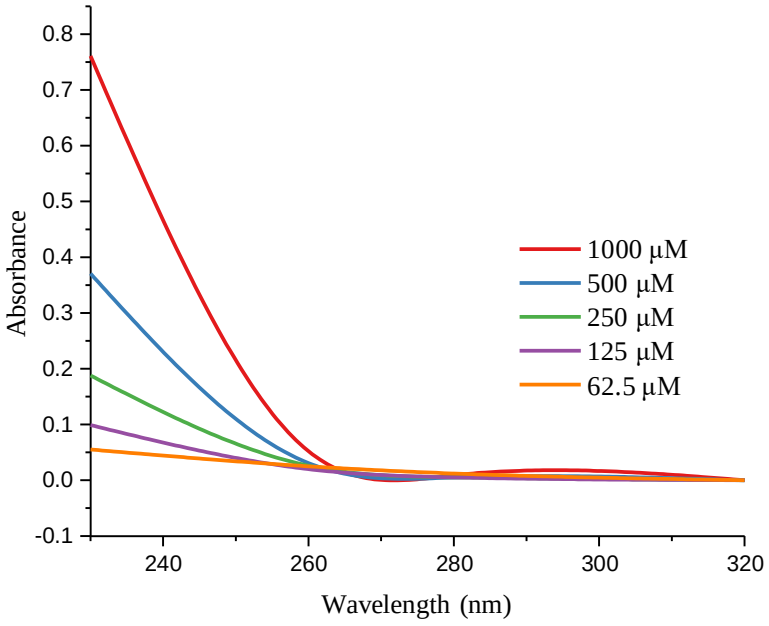

Figure 3.2 $CuCl_2$ UV-absorbance graph.

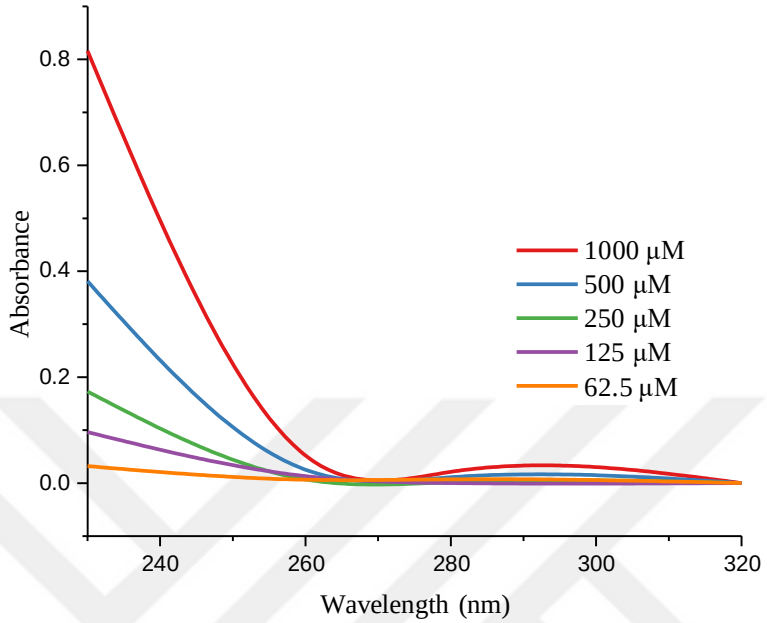

Figure 3.3 $CuSO_4$ UV-absorbance graph.

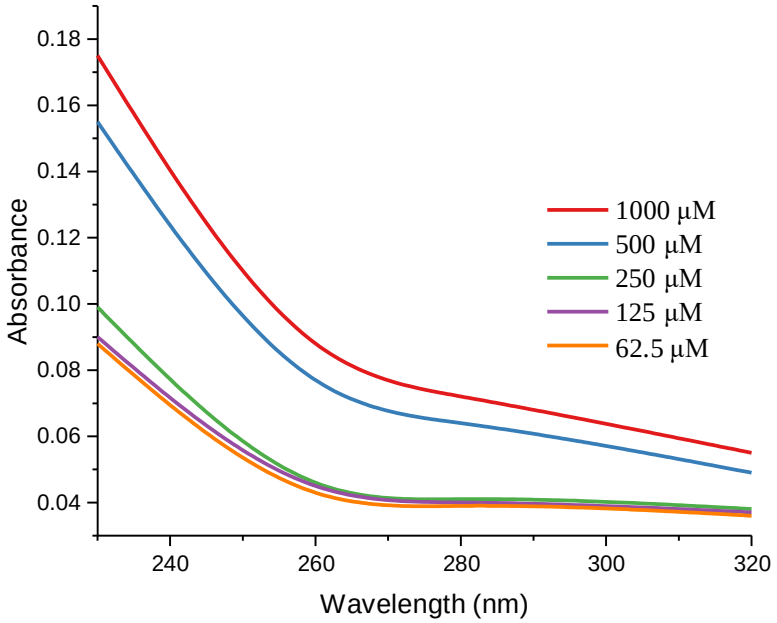

Figure 3.4 $CuCO_3$ UV-absorbance graph.

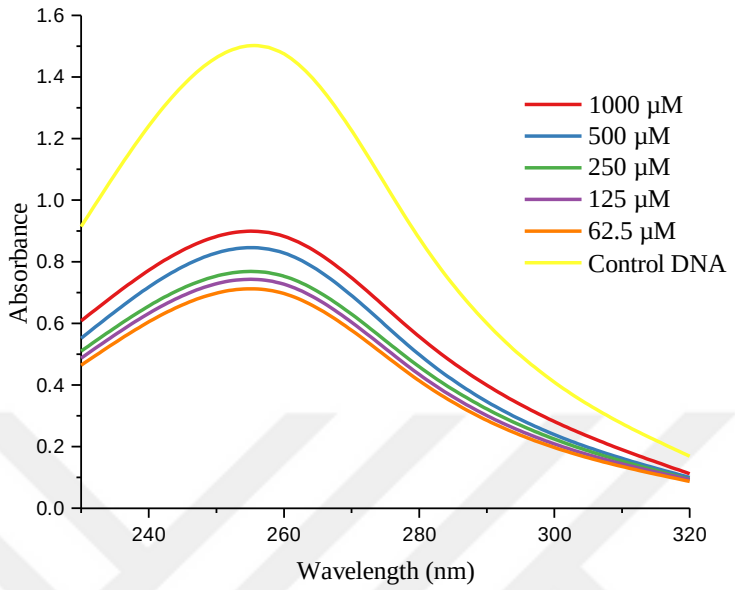

Figure 3.5 UV-absorbance spectrophotometry graph of DNA-$CuCl_2$ interaction in different concentrations.

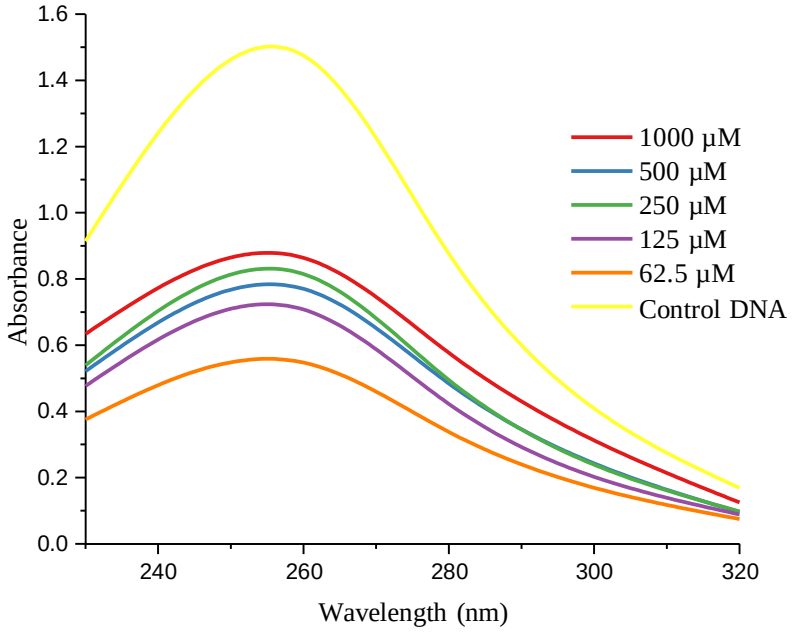

Figure 3.6 UV-absorbance spectrophotometry graph of DNA-$CuSO_4$ interaction in different concentrations.

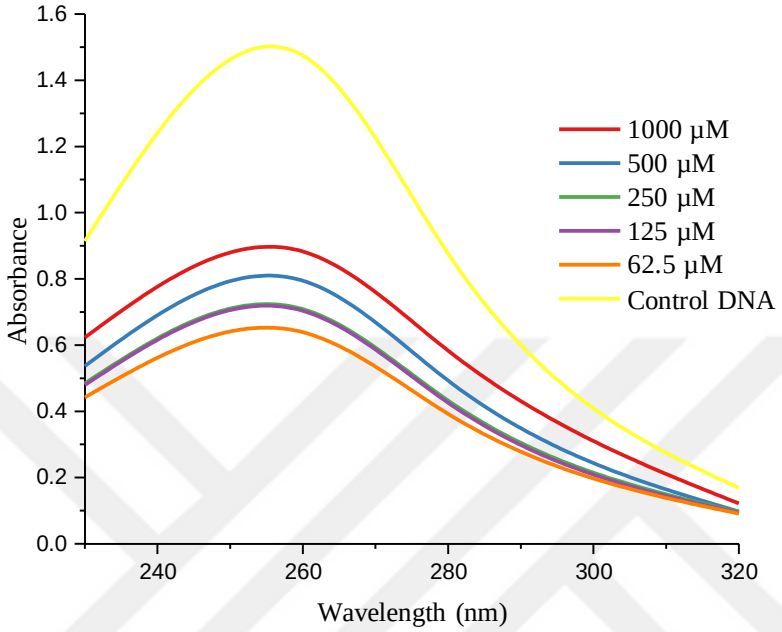

Figure 3.7 UV-absorbance spectrophotometry graph of DNA-$CuCO_3$ interaction in different concentrations.

3.3. Agarose Gel Electrophoresis

The gel image and band interactions obtained from the agarose gel electrophoresis study on the ct-DNA and the various concentrations of the copper compounds are represented in figure 3.8, table 3.2, figure 3.9, figure 3.10 and figure 3.11.

The obtained agarose gel electrophoresis data indicates that gel radiation (brightness) declined upon adding the copper compounds to the ct-DNA. The control had the brightest and highest band volume of 49.757. However, upon combining ct-DNA with the copper compounds, reduced brightness and reduced band volume was observed with respect to increment in copper compounds' concentrations. In other words, a concentration-dependent decrement in brightness and band volume was

observed. Also, copper (II) chloride at the concentration 1000 µM had the lowest brightening and lowest band volume of 29.762

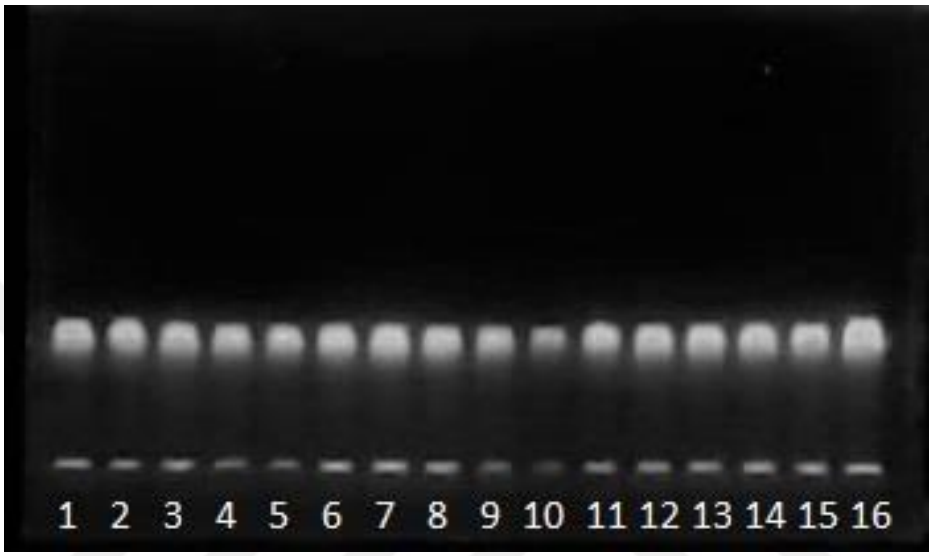

Figure 3.8 Image of gel and bands from ct-DNA combination with copper compounds. **1**: ct-DNA+62.5 µM $CuSO_4$, **2**: ct-DNA+125 µM $CuSO_4$, **3**: ct-DNA+ 250 µM $CuSO_4$, **4**: ct-DNA+500 µM $CuSO_4$, **5**: ct-DNA+1000 µM $CuSO_4$, **6**: ct-DNA+62.5 µM $CuCl_2$, **7**: ct-DNA+ 125 µM $CuCl_2$, **8**: ct-DNA+250 µM $CuCl_2$, **9**: ct-DNA+500 µM $CuCl_2$, **10**: ct-DNA+1000 µM $CuCl_2$, **11**: ct-DNA+62.5 µM $CuCO_3$, **12**: ct-DNA+125 µM $CuCO_3$, **13**: ct-DNA+250 µM $CuCO_3$, **14**: ct-DNA+500 µM $CuCO_3$, **15**: ct-DNA+1000 µM $CuCO_3$, **16**: Control.

Table 3.2 Agarose gel electrophoresis data (as band volume) obtained from ct-DNA and copper compounds combinations.

No.	Type	DNA Band Volume (Integer)	Area (mm^2)
1	ct-DNA+62.5 µM $CuSO_4$	40.139	27.7
2	ct-DNA+125 µM $CuSO_4$	39.539	27.7
3	ct-DNA+ 250 µM $CuSO_4$	38.204	27.7
4	ct-DNA+500 µM $CuSO_4$	37.818	27.7
5	ct-DNA+1000 µM $CuSO_4$	36.414	27.7
6	ct-DNA+62.5 µM $CuCl_2$	44.276	27.7
7	ct-DNA+ 125 µM $CuCl_2$	41.134	27.7
8	ct-DNA+250 µM $CuCl_2$	40.451	27.7
9	ct-DNA+500 µM $CuCl_2$	35.445	27.7
10	ct-DNA+1000 µM $CuCl_2$	29.762	27.7
11	ct-DNA+62.5 µM $CuCO_3$	42.979	27.7
12	ct-DNA+125 µM $CuCO_3$	42.859	27.7
13	ct-DNA+250 µM $CuCO_3$	41.701	27.7
14	ct-DNA+500 µM $CuCO_3$	41.004	27.7
15	ct-DNA+1000 µM $CuCO_3$	38.980	27.7
16	Control DNA	49.757	27.7

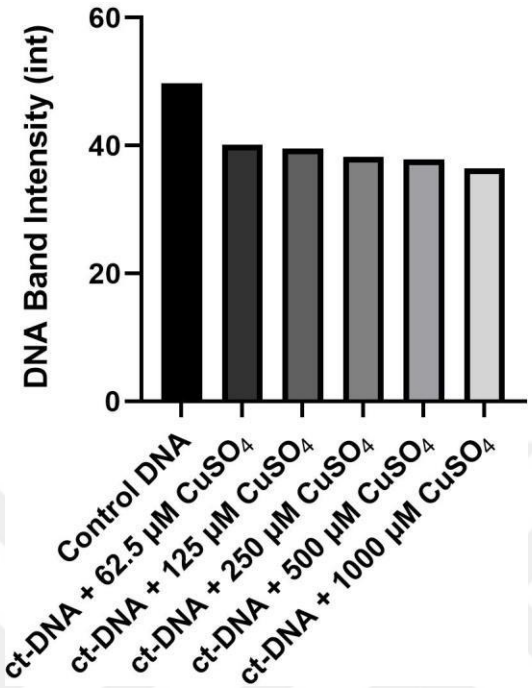

Figure 3.9 Band volumes of ct-DNA with the copper (II) sulphate pentahydrate.

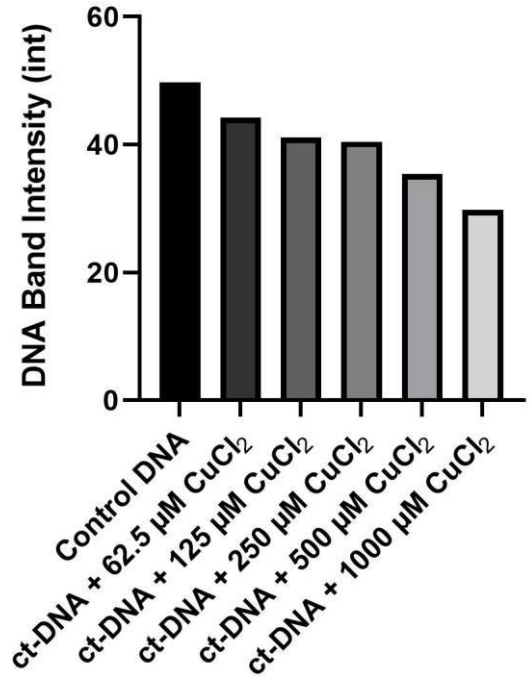

Figure 3.10 Band volumes of ct-DNA with the copper (II) chloride.

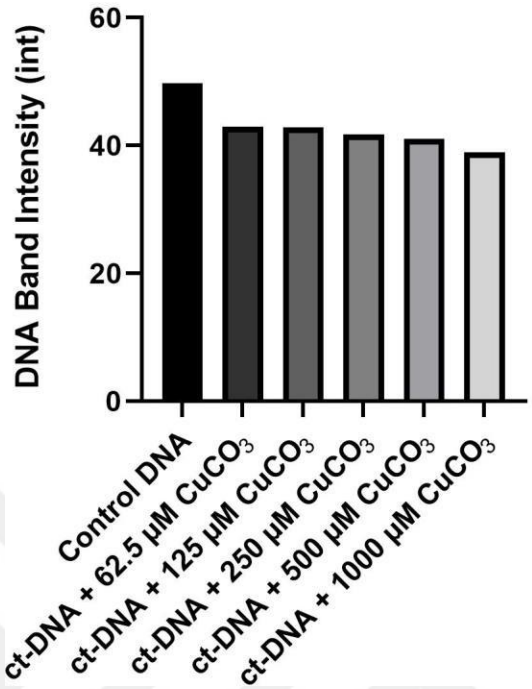

Figure 3.11 Band volumes of ct-DNA with the copper (II) carbonate.

3.4 Fluorescence Spectrophotometry

According to the results, although the genomic DNA did not give any fluorescence peak in the 400-700 nm wavelength range, just as the copper compounds. When combined with the fluorescent DNA dye fluorescent peaks were observed (Figures 3.12, 3.13, 3.14, 3.16, 3.17, 3.19, 3.20). The fluorescence study shows that the copper compounds have a fluorescent quenching effect, especially $CuCl_2$. The strongest hypochromic effect of $CuCl_2$ in the range of 400-700 nm was observed at 1000 µM concentration (Figure 3.15). $CuSO_4$ compound showed a hypochromic effect (400-700 nm) at concentrations above 62.5 µM. However, this effect was not proportional to its concentrations, and the strongest hypochromic effect occurred at 125 µM concentration (Figure 3.18). When the fluorescent spectrophotometry data were evaluated, it was determined that $CuCO_3$ did not interact with DNA at the concentrations examined within the scope of this study (Figure 3.21).

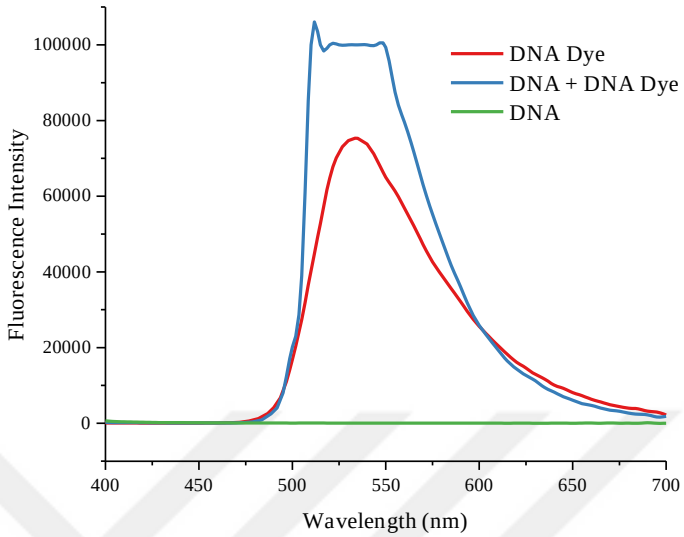

Figure 3.12 Fluorescence spectrophotometry measurement of DNA, DNA Dye, and DNA + DNA Dye.

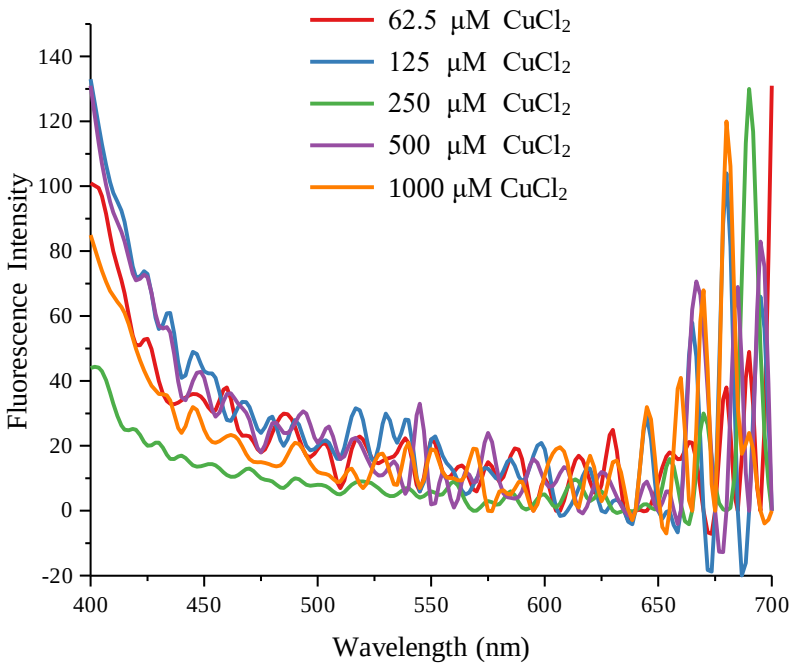

Figure 3.13 Fluorescence spectrophotometry measurement of only $CuCl_2$.

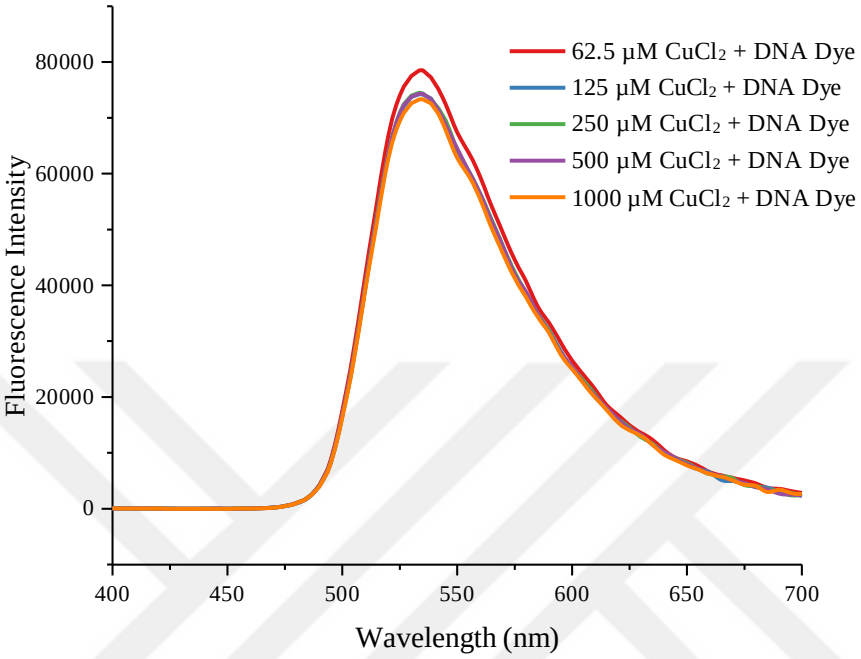

Figure 3.14 Fluorescence spectrophotometry measurement of $CuCl_2$ + DNA Dye.

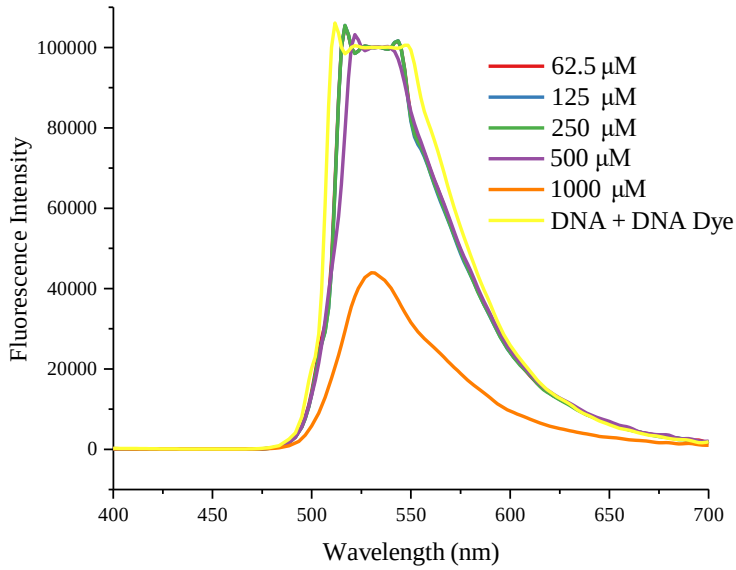

Figure 3.15 Fluorescence spectrophotometry measurement of ct-DNA + DNA Dye + $CuCl_2$.

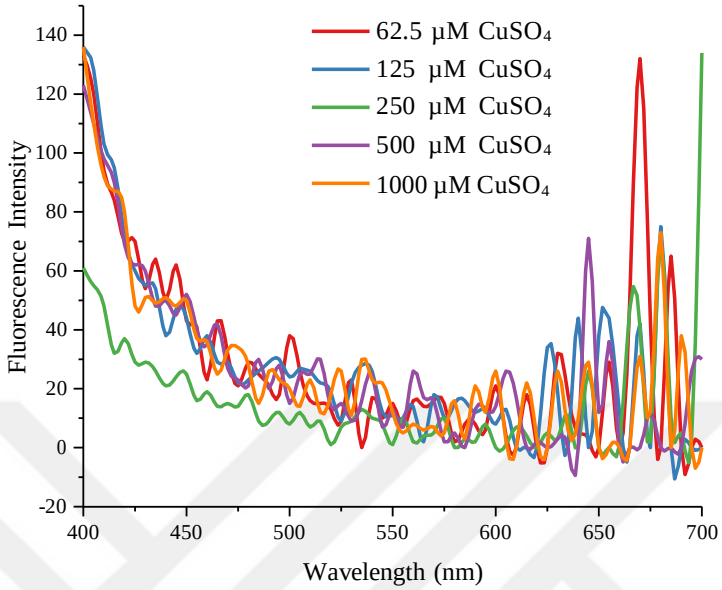

Figure 3.16 Fluorescence spectrophotometry measurement of only $CuSO_4$.

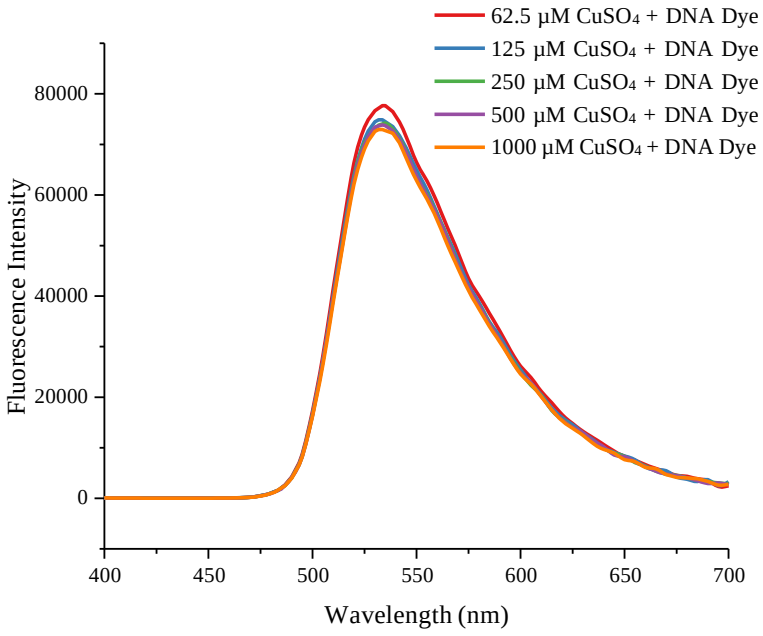

Figure 3.17 Fluorescence spectrophotometry measurement of $CuSO_4$ + DNA Dye (Control).

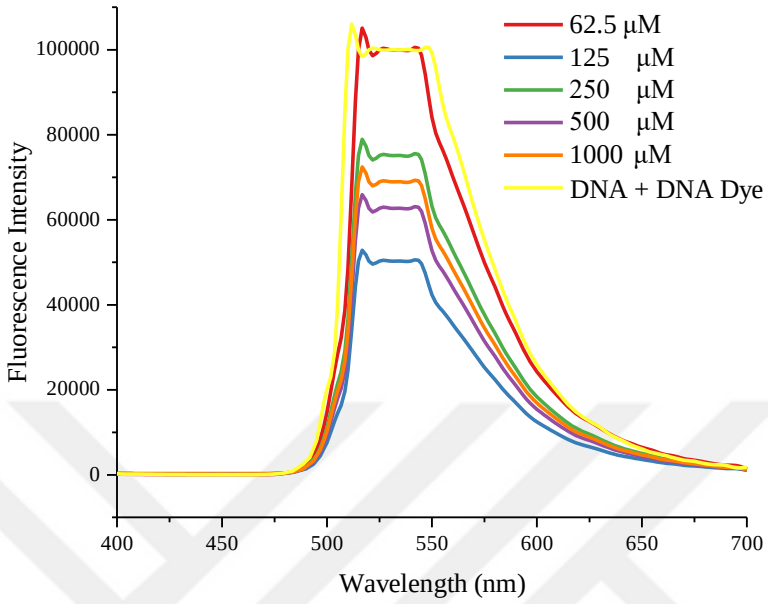

Figure 3.18 Fluorescence spectrophotometry measurement of ct-DNA + DNA Dye + $CuSO_4$.

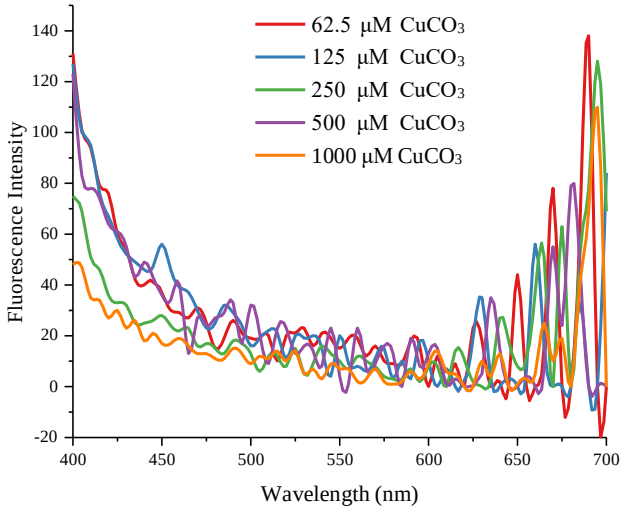

Figure 3.19 Fluorescence spectrophotometry measurement of only $CuCO_3$.

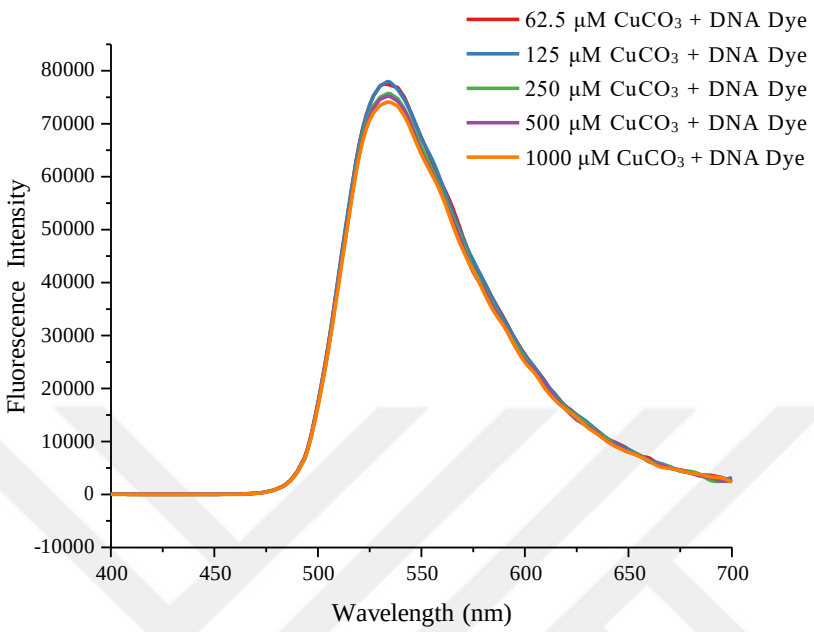

Figure 3.20 Fluorescence spectrophotometry measurement of $CuCO_3$ + DNA Dye.

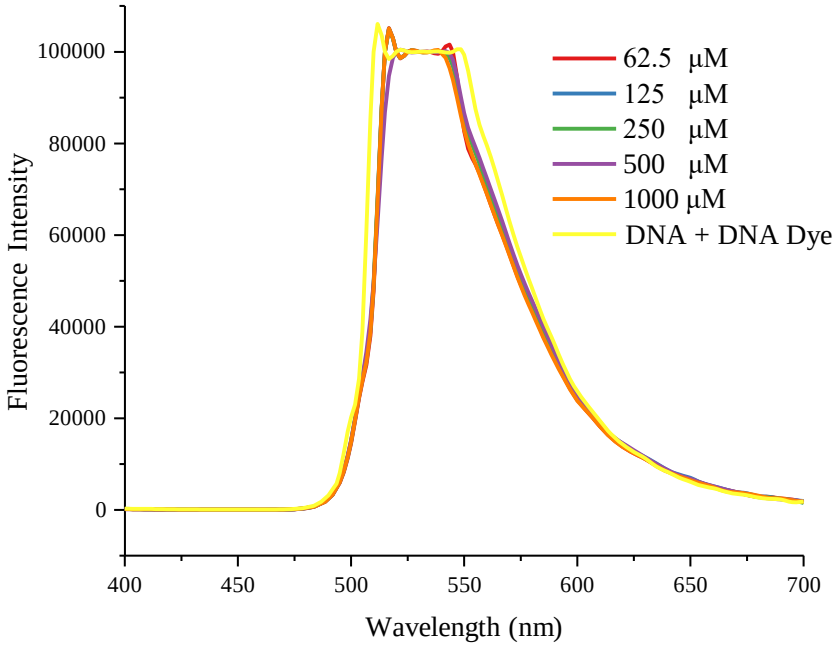

Figure 3.21 Fluorescence spectrophotometry measurement of ct-DNA + DNA Dye + $CuCO_3$.

4. DISCUSSION

Since DNA is the primary target of most pharmacological targets, especially anticancer agents, its interaction with binding molecules like metal compounds provides a clue of paramount relevance for the development of effective and safe metal-based therapeutic agents, chemotherapeutic agents (Qiao et al., 2011). Hence, the potential binding ability of the copper compounds: $CuSO_4$, $CuCl_2$ and $CuCO_3$ to ct-DNA were investigated by adopting UV-vis absorption spectroscopy. Electronic absorption spectroscopy is one of the many methods used to investigate the interaction of metals, their complexes, and their salts with DNA. When a binding molecule interacts with DNA by intercalation, it usually leads to hypochromism and bathochromism (red shift) of the absorption band due to the strong stacking interaction between the aromatic moiety of the binding molecule and the base pairs of the DNA (Chaveerach et al., 2010; Chen et al., 2011). The hypochromism extent is often consistent with the strength of the intercalative binding activity (Chen et al., 2011). Contrary, the results of this study indicate that the various copper compounds incited an increase in DNA absorption intensity at 260 nm in a concentration-dependent manner (figure. 3.5, 3.6, and 3.7). Thus, an increase in the concentrations of the copper compounds induced an increase in DNA absorption intensity (hyperchromism). This probably occurred owing to the shapeshifting of the DNA-double helix structure upon an increment of the copper compounds' concentrations (Chaveerach et al., 2010; Li et al., 1996).

The presence of hyperchromism is an indication that the copper compounds probably interacted with the ct-DNA via partial or non-intercalative binding mode, like, groove binding (Dey et al., 2010; Vijayalakshmi et al., 2000). This could be due to 1) the formation of hydrogen bonds between the oxygen element of the copper compounds and amine (-NH) groups of the nucleotide bases, and 2) the existence of an electrostatic force of interaction between the copper compounds and the negatively charged phosphate groups of the nucleotide bases. These findings are consistent with the findings of

Chaveerach et al. (2010), where two designed copper (II) complexes incited a hyperchromic effect on ct-DNA, hence, interacted with ct-DNA via non-intercalative mode. Nonetheless, these findings are not consistent with the findings of Liu et al. (2002). Liu et al. (2002) reported that on adding increasing concentrations of ct-DNA to the fixed concentrations of the macrocyclic copper (II) complexes (40 μM), the absorption spectra of ct-DNA manifested reduction, hypochromism and a red shift, thus, suggesting the presence of intercalative binding. Most planar metal complexes seem to interact with DNA by intercalation (Biver et al., 2004, Biver et al., 2008; Tan et al., 2009). Despite that, the thermodynamic and biological results obtained by Ruiz et al. (2010) in a study conducted to determine the binding modes of the copper thiosemicarbazone complexes; $[CuL]^+$ and $[CuL']^+$ to ct-DNA indicated that the complexes behaved as groove binders, which is similar to findings of this study.

The exhibition of the non-intercalative mode of binding by the copper compounds could be due to low copper compound/DNA ratios. In consistence with the results of this study, hyperchromism effect was observed at low $CuCl_2$ complexes/DNA ratios. This was attributed to the possible interaction of the copper complexes with DNA via external contact (electrostatic binding) or the $CuCl_2$ complexes partially uncoiling the DNA structure, thus, exposing the nucleotide bases of the DNA, hence the increment in absorption spectra. Conversely, at high $CuCl_2$ complex/DNA ratios, the copper complexes interacted with the DNA via intercalation as hypochromism and bathochromism were observed (Meenongwa et al., 2016).

The agarose gel electrophoresis technique, which is also one of the methods used in the examination of intermolecular interactions was employed in this study to investigate the interaction between the copper compounds and DNA (Xie et al., 2015). The DNA binding and cleavage activities of the copper compounds were studied by determining the conversion of the ct-DNA to the various forms, namely; form I (which is the supercoiled form), form II (nicked circular), and form III (the linear form) based on their mobility rates on the gel. Thus, agarose gel electrophoresis was adopted in the studying of their cleavage activities. Form I exhibit a relatively fast rate of migration, and form II is known to be the bulkiest hence, it has the slowest rate of migration. Form III usually migrates between form I and form II. However, form II and form III were not

observed in this study (Figure.3.8). When only form I is formed, then the supercoiled DNA was not cleaved, if one strand is cleaved, the supercoiled DNA relax to form the slower-moving circular form (form II). The formation of the linear form (form III) indicates the cleavage of the double strands of DNA. Hence, extrapolating from the gel electrophoretic results of this study, it could be concluded that there was no DNA cleavage since form II and form III were not formed (Liu et al., 2002; Meenongwa et al., 2016).

In spite of the absence of DNA nicking, the copper compounds induced a decrease in DNA band intensities. An increase in the concentrations of the copper compounds incited a decrease in band intensities and this could be as a result of the binding effect of the copper compounds on DNA. Although all the copper compounds bind to the DNA, $CuSO_4$ seemed to be a good binder, because it induced relatively lower band intensities at 62.5 μM, 125 μM, 250 μM and 500 μM concentration. Conversely, $CuCl_2$ at the concentration of 1000 μM incited a relative extremely lower band intensity. This probably indicates that $CuCl_2$ binds effectively to DNA at a higher concentration. The decrement in band intensities instigated by the copper compounds could be an indication that the copper compounds interacted with the ct-DNA through intercalation.

Undeniably, DNA has a very weak fluorescence intensity. Also, no fluorescence was observed for the copper compounds. Therefore, directly conducting a fluorescence emission on the ct-DNA and the copper compounds would have yielded no results. The standard and common method for enhancing fluorescence is the use of ethidium bromide (EB). However, EB is toxic and carcinogenic, therefore, in this study, it was replaced with SafeView nucleic acid stain/dye, which is also an intercalator. In other words, it is a safer alternative for EB (Shen et al., 2011; Şenol, 2020; Şenol, 2021).

The results obtained from the fluorescence spectrophotometry indicate that the fluorescence intensity of the SafeView stain decreased upon addition of the copper compounds, except for $CuCO_3$, which did not show any macroscopic differences. That is to say, the copper compounds quenched the fluorescence intensity of the SafeView. Its fluorescence intensity decreased as the concentration of the copper compounds

increased. Hence, the copper compounds exhibited hypochromism. SafeView is an intercalator and the fluorescent specie, so, the decrement in its fluorescence intensity means that the copper compounds probably replaced it, they possibly bind to the ct-DNA by intercalation. Consistent to these results, hypochromism coupled with bathochromism (intercalation) was observed upon adding Cu (II)/Neotrien to ct-DNA which was previously saturated with EB. The addition of Cu (II)/Neotrien decreased the fluorescence intensity of the EB and this decrement was associated to Cu(II)/Neotrien replacing the EB (Biver et al., 2004). Also, copper (II) complexes have been reported to have appreciably decreased the fluorescence intensity of EB by competing with EB for binding to DNA. Again, according to the UV-VIS spectrophotometry results of that study, two of the copper (II) complexes showed decrease in absorption intensity. Thus, it was concluded that they may bind to the DNA through partial intercalation.

On a contrary, inferencing from the UV-vis spectrophotometry results of the present study, where the copper compounds exhibited hyperchromism, indicating that they might have interacted with the ct-DNA by non-intercalative binding (groove binding). It is deduced that the instigation of decrement in the fluorescence intensity of the SafeView (hypochromism) by the copper compounds does not necessarily mean they are intercalators. The copper compounds via groove binding to the ct-DNA might have manifested SafeView fluorescence quenching by releasing some SafeView molecules from the SafeView-ct-DNA system. Similarly, copper (II) complex exhibited EB fluorescence quenching through the groove binding mode by releasing some EB molecules from the EB-DNA system (Dey et al., 2010).

5. CONCLUSION and RECOMMENDATION

When the data obtained with the three techniques used in the present study are evaluated together, it can be concluded that the copper compounds investigated by gel electrophoresis do not cleave DNA and they reduce the band intensity of DNA as an indication of their binding to the DNA molecule. Also, by the UV-VIS absorbance technique which does not require the use of a DNA stain/dye, groove binding findings were observed in the spectrophotometric scanning (220-320 nm). On the other hand, it was observed that the copper compounds could exhibit a tendency to replace the nucleic acid dye/stain molecules when fluorescent spectrophotometry within the preferred wavelength range (400-700 nm) was conducted. Consequently, the copper compounds of the present study prepared at certain concentrations bind to DNA, most probably by non-intercalative mode. In this regard, they could have the potential to be used in the development of new therapeutic agents. Thence, conducting further studies on the interaction of the copper compounds with DNA, their possible DNA uncoiling activities, and also the investigation of these compounds in cancer cell lines will provide useful results.

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