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

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Abstract **–**UV-absorbance spectrophotometry and agarose gel electrophoresis techniques were employed to investigate the interaction of genomic DNA with some copper compounds ($CuSO_4$, $CuCO_3$, and $CuCl_2$) at the concentrations; 1000 µM, 500 µM, 250 µM, 125 µM, and 62.5 µM. When the UV-spectrophotometry data were examined within the wavelength range of 220-320 nm, the hyperchromic effect of $CuCl_2$ was evaluated to be proportional to its concentrations. Within the wavelength range, the copper compounds produced their strongest hyperchromic effect on the DNA at 1000 µM. According to the agarose gel electrophoresis findings, the copper compounds investigated decreased DNA band intensity as their concentrations increased. It was observed that the most significant decrease in band intensity was caused by $CuCl_2$ at 1000 µM. Also, no cleavage in the genomic DNA was caused by the copper compounds within the concentration range of this study (62.5-1000 µM). Consequently, the copper compounds bind to DNA, most probably by non-intercalative mode. Thus, 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.

Keywords – Genomic DNA, Copper Compounds, DNA-Metal Interaction, UV-Absorbance, Fluorescence Spectrophotometry, Agarose Gel Electrophoresis

I. INTRODUCTION

Despite the importance of some metal ions in the maintenance of DNA stability and structure, some are also known to be mutagenic and carcinogenic. Pieces of evidences about the mutagenicity, and carcinogenicity of metal ions were obtained from several research studies conducted on the interactions between some d-block elements and DNA. Non-physiological transition metals interacting with DNA can cause irreversible cell damage and eventually, apoptosis [1]. Modifying or inhibiting the activity of a cell's DNA is essential medical research that promotes 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 [2]. Considering therapeutic agents like the anticancer agents, DNA cleaving agents have attracted the attention of scientists in the field of molecular biology and drug development [3].

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 [4,5]. In response to these toxic effects, alternate metal complexes such as copper complexes (Cu 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 as therapeutic agents [4].

Cu is a versatile transition metal relevant for all forms of life. Cu has a vast variety of functions including; angiogenesis, antioxidant, and as a cofactor for several enzymes such as cytochrome oxidase [6]. 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 [7]. Metal complexes including copper complexes have shown varying efficiencies in hydrolysing RNA and phosphate diester [4,8,9].

There is a surge in interest in research on interaction of nucleic acids 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 [4,10]. In spite of this, the role of metal ions and metal complexes in binding with nucleic acids has been less studied [11]. In view of that, this study aimed to investigate the interaction of genomic DNA (calf thymus DNA, ct-DNA) with $CuSO_4$, $CuCO_3$, and $CuCl_2$ via UV-spectrophotometry, and agarose gel electrophoresis, to serve as potential candidates for drugs development.

II. Materials and Method

Calf thymus tissues were collected from the municipal slaughterhouse of Kırıkkale-Turkey and in a cold chain, were transported to the molecular laboratory of the Kırıkkale University-Turkey for DNA isolation. Prior to that, Ethical approval was obtained from the Kırıkkale University Animal Experiments Local Ethics Committee (55762/02.11.2021). Wizard® Genomic DNA Purification Kit (A1120, Promega, USA) was used for the DNA isolation. Its concentration and purity at Å 260/280 were determined with a micro-drop spectrophotometer (Multiskan GO, Thermo Scientific, USA**).**

A. UV-Absorbance Spectrophotometry

The absorbance spectra of the prepared copper compounds and the isolated DNA were measured with the micro-drop spectrophotometer (Multiskan GO, Thermo Scientific, USA**)**. After that, the various concentrations of the copper compounds (62.5 µM, 125 µM, 250 µM, 500 µM, and 1000 µM) were combined with the DNA at a ratio of 1:1 (5 µl of DNA and 5 µl of each concentration of the copper compounds) and incubated at 37 ℃ for 60 minutes. Subsequently, the absorbance spectra of the combinations were also measured. All UV-spectrophotometric measurements were done within 220-320 nm wavelength range (λ range).

B. Agarose Gel Electrophoresis

With a horizontal electrophoresis system (nanoPAC-300, Cleaver Scientific, UK), the agarose gel electrophoretic study was conducted. A gel density of 1.5 %, an electric current of 100 Volts, and a running time of 1.5 hours were used in the study upon conducting preliminary studies. The combinations of DNA with the various concentrations of the copper compounds were again done at a 1:1 ratio (5 µl of DNA and 5 µl of each concentration of the copper compounds) and incubated at 37 ℃ for 60 minutes. Following the incubation process, the 10 µl mixture of DNA and copper compound were mixed with 2 µl of DNA loading dye and 2 µl of Ez-vision dye before loading into the gel wells. After the loading process, electrophoresis was conducted. UV transilluminator (WiseUV WUV-L2O, Germany) was used to visualize the bands that were formed in the gels. The gel photographs were recorded using Gel Doc™ EZ, Bio-Rad, Turkey and the DNA band intensities were evaluated with Image lab 6.0, Bio-Rad, Turkey.

III. RESULTS

The DNA isolated from the calf thymus tissue with Wizard® Genomic DNA Purification Kit (A1120, Promega, USA) had a purity of 1.84 at Å 260/280. Hence, was suitable for the UV-spectrophotometry, and agarose gel electrophoresis study.

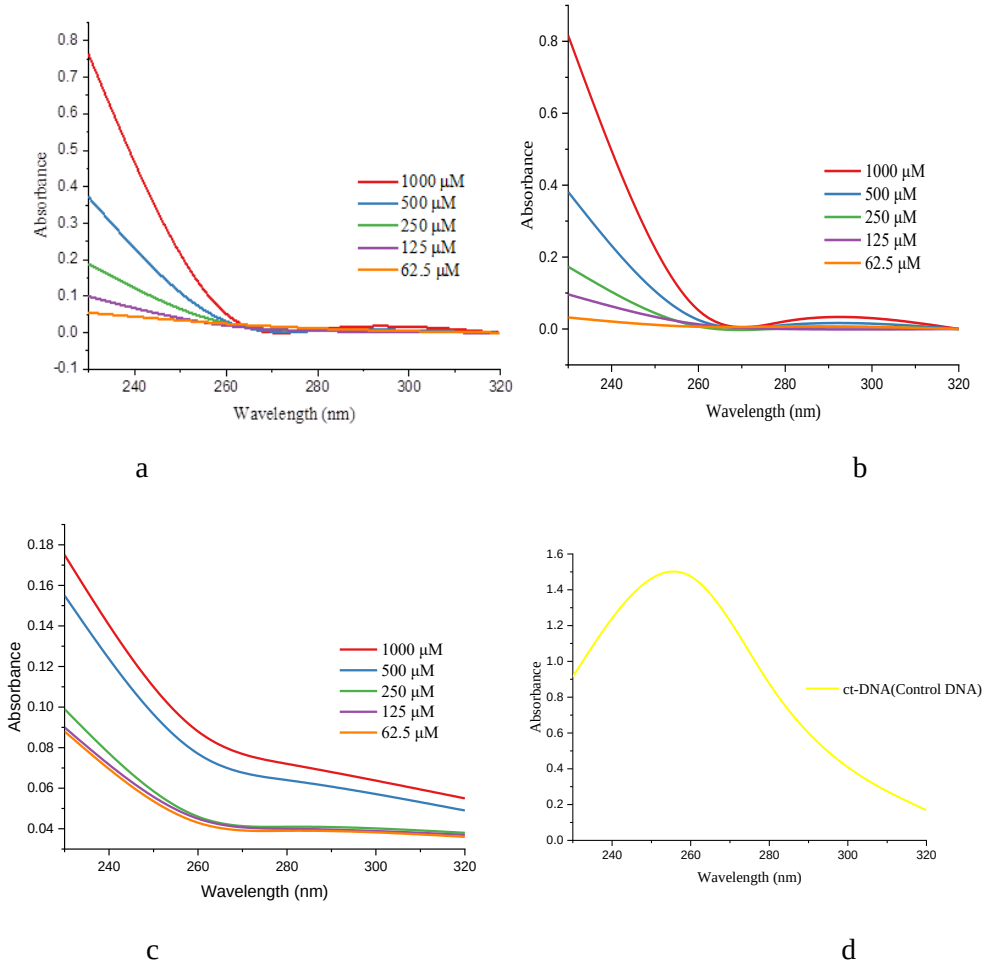

Figure 1. UV-absorbance spectra of $CuCl_2$ (a), $CuSO_4$ (b), $CuCO_3$ (c) and ct-DNA (d)

A. UV-absorbance Spectrophotometry

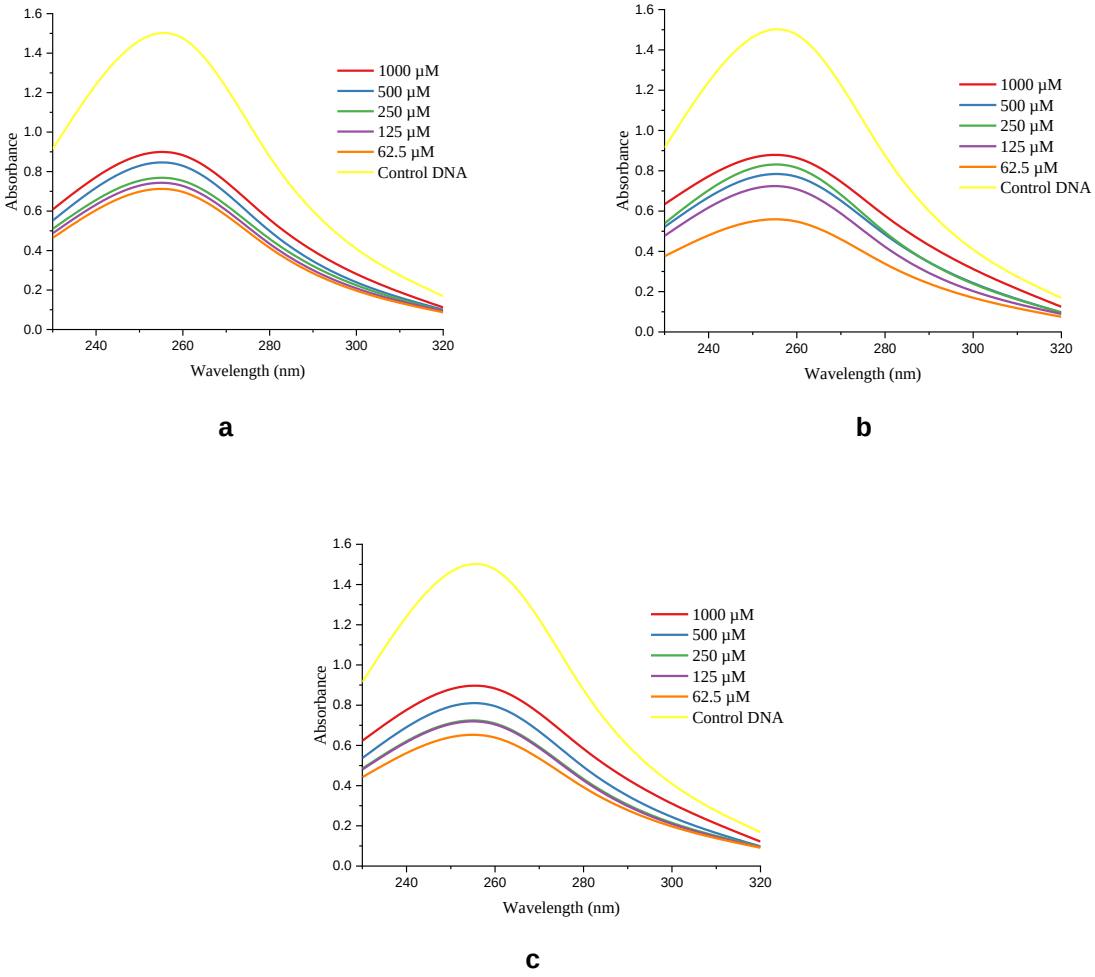

Figure 2. UV-absorbance spectrophotometry graph of DNA-$CuCl_2$ (a), DNA-$CuSO_4$ (b), and DNA-$CuCO_3$ (c) interaction in different concentrations.

B. Agarose Gel Electrophoresis

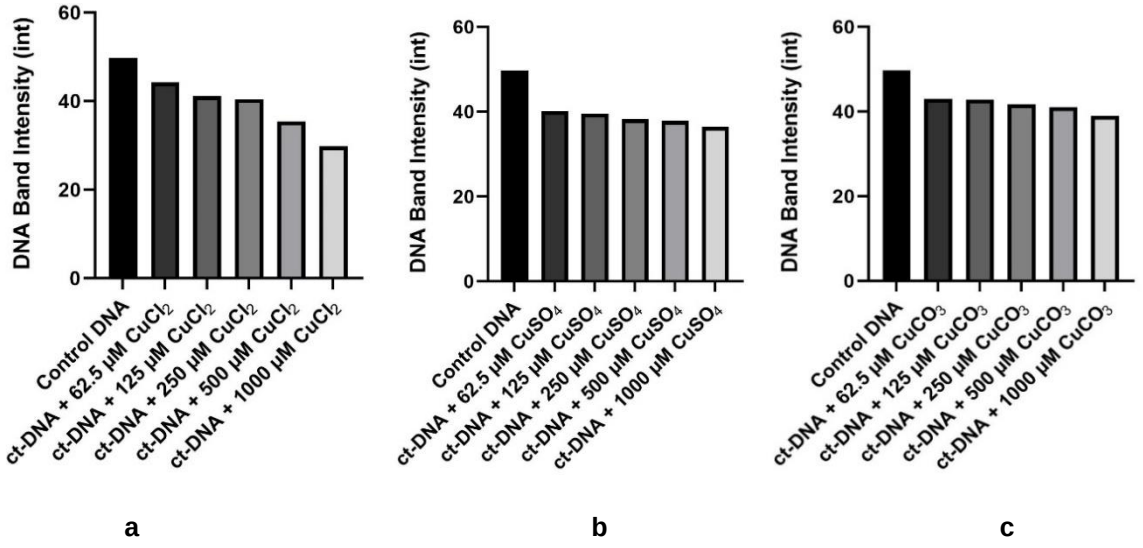

Figure 3. Band volumes of ct-DNA with $CuCl_2$ (a), $CuSO_4$ (b), and $CuCO_3$ (c)

IV. DISCUSSION

UV-absorbance Spectrophotometry

According to the obtained data of the UV-absorbance spectrophotometry, as expected, the copper compounds did not give absorbance peaks at 260 nm (Fig. 1). However, in addition to the control DNA, it was observed that the combinations of ct-DNA with the various concentrations of the copper compounds gave wave peaks at 260 nm (Fig. 2). UV 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 [12,13]. Contrary, the results of this study indicate that the copper compounds incited an increase in DNA absorption intensity at 260 nm in a concentration-dependent manner. Thus, an increase in the concentrations of the copper compounds induced an increase in DNA absorption intensity (hyperchromism). Perhaps, this occurred due to a shapeshifting of the DNA-double helix structure upon an increment of the copper

compounds' concentrations [12,14]. The presence of hyperchromism indicates that the copper compounds might have interacted with the ct-DNA via non-intercalative binding mode (groove binding) [15,16].

Agarose Gel Electrophoresis

The data of the agarose gel electrophoresis indicate a decline in gel radiation (brightness) upon adding the copper compounds to the ct-DNA. The control DNA (sample 16) had the brightest and highest band volume. However, the combinations (ct-DNA + copper compounds) had reduced brightness and reduced band volume with respect to increase in copper compounds' concentrations (concentration-dependent). Also, copper (II) chloride at the concentration 1000 μM (sample 10, in Fig. 5) had the lowest brightening and lowest band volume (Fig. 5). 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. 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 (Fig. 5).

The increase in copper compounds' concentrations incited a decrease in band intensities and this could be due to 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 concentrations (Fig. 6). On a contrary, $CuCl_2$ at the concentration of 1000 μM incited a relative extremely lower band intensity. This probably is an indication that $CuCl_2$ binds effectively to DNA at a higher concentration. Although there was no DNA nicking, the copper compounds decreased the DNA band intensities.

V. CONCLUSION

Extrapolating from the data obtained in this study, it can be concluded that the copper compounds investigated do not cleave DNA and they can bind to DNA molecule (due to the reduced band intensity of DNA). Also, by the UV-absorbance technique, groove binding findings were observed within the wavelength range 220-320 nm. Consequently, the copper compounds of the present study prepared at the aforementioned 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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