An-Najah National University

Faculty of Graduate Studies

In Vitro Evaluation of Anticancer Activity for Novel Family of Synthetic Copper/Polyamines Complexes

By

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Dedication

I dedicate my thesis to my family and many friends. A special feeling of gratitude goes to my loving parents, Mustafa and Tamam Mansour, who have raised me to be the person whom I am today and whose words of encouragement, push for tenacity ring in my ears. Thank you for all unconditional love, guidance, care and support that you have always given me. To my brothers Khaled, Husam, Abdulsalam and Mohammad you are all so special thank you for being there with me all the time. To my soul mate my husband Rasheed for his patience and encouragement. To my precious, my little angel, my daughter, Yara, thank you for being in my life.

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الإقرار

أنا الموقعة ادناه مقدمة الرسالة التي تحمل العنوان :

_In Vitro_Evaluation of Anticancer Activity for Novel Family of Synthetic Copper/Polyamines Complexes

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Declaration

The work provided in this thesis, unless otherwise referenced, is theresearcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Date: التاريخ: ١٠/٢/٢٠١٥

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List of Abbreviations

C1	Complex no.1
C2	Complex no.2
C3	Complex no.3
C4	Complex no.4
C5	Complex no.5
C6	Complex no.6
C7	Complex no.7
Cu	Copper
dipn	dipropylenetriamine
DMEM	Dulbecco's modified Eagle's medium
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbant assay
Hct	Colon cancer cell line
HepG2	Hepatic cancer cell line
IC50	Lethal dose which kills 50% of cell population
L6	Human skeletal muscle cell line
MD	Menkes disease
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PC3	Prostate cancer cell line
ROS	Reactive oxygen species
RPMI	Media was developed by Moore at Roswell Park Memorial Institute
TSCs	Thiosemicarbazones
UV	Ultra Violet
WD	Wilson disease
µl	Microliter

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Abstract

The cancer mortality and morbidity rate is rising all over the world in developed countries and in developing ones which make cancera major health problem. Many researches and efforts have been made in order to cure cancer, stop its spread or even to contain its damage. Chemotherapy is widely used to treat cancer but its side effects and toxicity are a major concern. One of the most significant discoveries in cancer chemotherapy is cisplatin which is very effective in treating different types of cancer especially testicular cancer which has a cure rate exceeds 90%. However, the treatment is limited because of several side effectsin addition to inherit and acquired resistance that limits its usage too. These drawbacks have motivated extensive search for other active antitumor metal- based complexes with improved pharmacological properties.

This study investigates the anticancer activity of copper polyamines complexes. Seven complexes were prepared and screened *in vitro* for both cytotoxic and cytostatic activities against four cell lines (human muscle cells as normal control: L6 cell line, colon cancer: Hct cell line, prostate cancer: PC3 cell line and liver cancer: HepG2 cell line) using MTT test. Results shows that complex 1 and complex 3 induced cytotoxic effect on

Hct (IC50: 0.03, 0.015), PC3 (IC50: 0.02, 0.015) and HepG2 (IC50: 0.015, 0.015) cell lines at nontoxic level compared to its effect on L6 cell line (IC50: 0.07). Also complex 7 induced cytotoxic effect on HepG2 cell line (IC50: 0.07) at nontoxic level compared to its effect on L6 cell line (IC50: 0.09). However, for cytostatic effect complex 1 and complex 3 induced cytostatic effect on PC3 and HepG2 (IC50: 0.015) cell lines at nontoxic level compared to its effect on L6 cell line (IC50: 0.02), also complex 4 and complex 5 induced cytostatic effect on HepG2 cell line (IC50: 0.13, 0.09) at nontoxic level compared to its effect on L6 cell line (IC50: 0.18, 0.1).

These complexes exhibited promising antiproliferative activity against tested cell lines which indicates that these complexes have anticancer effect at nontoxic concentrations.

Chapter One

Introduction

1. Introduction

1.1 General background

Cancer has become a major cause of death around the world among all other non-communicable diseases, especially in low- income and middle-income countries which tolerate now about 80% of the world load of such diseases. As the world population is growing UN predicts that global population will reach 8.3 billion by 2030[1] and that's making the growth an aging will be the maximum in the low-income and middle-income countries. These alterations transform to a predicted global load of 20.3 million new cancer cases by 2030 compared with 12.7 million cases in 2008, and a predicted 13.2 million cancer related deaths worldwide by 2030 up from 7.6 million in 2008 [2]. With these growing numbers efforts are being made with the aim to prevent, control and treat cancer through various research activities.

Canceris a set of malicious diseases that may affect various parts of the body which characterized by uncontrolled cell growth that could form masses of tissue called tumors. Such masses may have the capability to invade the adjacent organs or even proliferate through the body which called metastasis[3].

There are two types of tumors benign and malignant. Benign tumors are usually localized and do not invade nearby tissue and organs also it could be removed while usually don't need to be removed, while malignant

tumors -which the term cancer refer to it- grow rapidly and invade nearby tissue and organs of the body.When tumor cells reaches the blood stream or lymphatic system it spreads all over the body and this process called metastasis[4].

Causes of cancer could be a combination of both internal factors such as inherited mutations, hormones and immune conditions. And environmental acquired factors such as radiation, smocking, diet and infectious agents [5]. Tumors are variant and diverse but they all have the capability of uncontrolled growth unlike the normal tissue. This characteristic could be used by researchers and scientists to find out how to differentiate between normal cells and tumors and how to target them specifically [6].

Cancer can treated by surgery, radiation, chemotherapy, hormone therapy and biological therapy [7]. Nowadays the conventional way to treat cancer consists of surgery in combination with radiation or chemotherapy[8]. However the method of treatment depends on the location and the progress of the disease. According to studies the most common cancers types in men are prostate, lung and liver, while in women either breast or cervical cancer was the most common diagnosed neoplasm [1].

1.2 Liver cancer

Known as hepatocellular carcinoma which is considered the 5^{th} lethal cancer worldwide and the 3^{rd} most common cause of death due cancer in the globe[9, 10]. This type of cancer is very aggressive and its lethality

came from the resistance to the existing anticancer agents, lack of surgical biomarkers and the implied liver diseases that limits the use of chemotherapeutic drugs[11].

Hepatocellular carcinoma is usually associated with chronic liver diseases such as cirrhosis, hepatitis B and C, viral infection, hemochromatosis and inherited metabolic disorders [12, 13].

In spite the expansion of molecular targeting therapies, the most effective hepatocellular carcinoma treatments are still liver resection, transplantation and locoregional therapy[12].

1.3 Colon cancer

Known as colorectal cancer one of the most common cancers worldwide and considered the 2nd leading cause of death via cancer [14]. This type of cancer usually evolved from polyps and pre-neoplastic bumps which arise from the colon lining with combination of genetic mutations[15, 16].

Many studies on colorectal cancer shows that diet is the key role in this type of cancer and it could be prevented by adjusting the quality of food intake which participates directly in preventing and treating colorectal cancer [14,17].

The cure of colorectal cancer via surgery is high up to 90% if detected in early stages, the problem is the best screening method is colonoscopy which considered invasive and has low popularity among population, so

many effort are being made to develop less invasive method with higher sensitivity[14,18].

1.4 Prostate cancer

Prostate cancer considered as the most common malignant neoplasm diagnosed and the 3rd leading cause of death related to cancer among men in developed countries [19, 20]. It causes about 30,000 deaths per year in United States [21].

Like other types of cancer, prostate cancer evolves from accumulation of somatic, genetic and epigenetic changes that inactivate tumor suppressor genes and activate oncogenes[22, 23]. The most common risk factors for developing prostate cancer are advanced age, family history, race and diet [19]. In early stages of prostate cancer prostatectomy is the cure but in metastatic stages chemotherapy is the best method to be used, also external beam radiation is one of the standard treatments of prostate cancer[24].

1.5 Chemotherapy as a systematic cancer treatment

Chemotherapy is a systemic treatment using chemical substances to cure cancer or inhibit the tumor growth and extend the patient life or to reduce the disease symptoms. Chemotherapy may use alone or in combination with radiation therapy, surgery and radiotherapy. As chemotherapy a powerful tool to treat cancer is still a major defy to cancer cause such a highly robust drug can be toxic and less than 1% of the injected drug molecules can be delivered to the target cells while the rest may damage

the other healthy cells and tissue particularly bone marrow, epithelial tissue, reticuloendothelial system and gonads [25]. This toxicity occurred because many current chemotherapeutic drugs have low selectivity and cannot distinguish between normal cells and tumor cells so it kills both normal and malignant cells [26].

One of the major side effects of chemotherapy is drug resistance which may occur after long time of using the chemotherapeutic agent [27], and that leads to the continuous search for new anti-cancer agents that offers both selectivity of malignant cells and counter act the mechanisms of resistance produced by malignant cells. One of the most significant discoveries for cancer chemotherapy in the 20^{th} century is the cisplatin [28].Cisplatin is very effective in treating different types of cancer particularly testicular cancer which has a cure rate exceeds 90% [29]. However, the treatment is limited by several side effects including nephrotoxicity, emetogenesis, hepatotoxicity and neurotoxicity [30]. Also cisplatin is not orally bioavailable and both the inherited and acquired resistance limits its usage [31]. These obstacles have motivated the extensive search for other active antitumor metal-based complexes with improved pharmacological properties. Metal complexes may undergo both which allow them to participate in a variety of biological redox chemistries and interact with different biological substrates [32, 33].

1.6 Anticancer activity of metal ions

Medicinal inorganic chemistry is an important field of chemistry that provide the chance to design novel metal-based therapeutic and diagnostic agents not readily available to organic compounds, also it can utilize the unique properties of metal ions to design new drugs[34]. The use of metals in medical application could be traced back to 5000 years [35]. The modern effort and search for metal based treatments is triggered by the discovery of cisplatin in 1965, this discovery has led to a new unexplored world of metal based therapeutic agents which have different kinetics and mechanism of action from those in conventional organic drugs [36].

Metal ions have essential roles in osmotic balance, signal transduction, functions of various proteins, nucleic acids, lipids and glycans. In fact, about 30-40% of all proteins contain metal ions and metalloenzymes catalyze some of the most mysterious reactions found in nature such as nitrogen fixation, water splitting and methane oxidation[37].

An important feature of metal ions is that they easily lose electron from their elemental state to form a positively charged ions which make it soluble in biological fluids. In this cationic form that metal become active in biological reactions, because of its electron deficient positively charged state while most of biomolecules such as proteins and DNA are electron rich negatively charged.The attraction between these opposing charges leads to a general tendency for metal ions to bind to and interact with biomolecules [38]. Metal complexes with labile ligands known with its

tendency to do ligand substitution reactions with biomolecular targets, they can bind to nitrogen, sulfur or selenium atoms of histidine, cysteine or selenocysteine residues in protein which give eventually the desired therapeutic effect [39].

Metal ions have been used in chemotherapy as anticancer agents to target the DNA directly such as cisplatinwhich covalently bond to DNA and forming adducts that lead to interstrand and intrastrand crosslinks which causes significant distortion of helical structure and result in inhibition of DNA replication and transcription which finally trigger cell death [40-42]. In order to overcome cisplatin toxicity and tumor resistance efforts are being made to find new metal based anticancer agents which are capable to overcomethese problems and maintain its efficacy. Nowadays, there are many other metal based drugs have different mode of actions such as non-covalent DNA interactions, protein targeting such as histone deacetylase, telomerase, topoisomerase and protein kinases, numerous metal complexes mediate cytotoxic activity through enzyme inhibition, others using metal as scaffold rather than as reactive center , superoxide dismutase mimic and produce oxygen radicals [8, 30, 34, 39].In order to find metal complexes with less side effects and similar or better cytotoxicity, a wide variety of metal complexes based on gold, ruthenium, manganese, iron, titanium, gallium, germanium, palladium, cobalt and copper are being intensively studied as platinum replacements[30, 33, 36, 38, 43, 44].Moreover, copper (II) based complexes appear to be very promising candidate for anticancer

treatment and this idea is supported by a lot of studies and researches that characterize the synthesis and cytotoxic activities of numerous copper (II) complexes [45-48]

1.7 Copper complexes as anticancer agent

Copper is found in all living organisms and is an essential trace element in redox chemistry, growth and development[49]. It has an important role inseveral enzyme function and proteins, implicated in energy metabolism, respiration, DNA synthesis, cytochrome oxidase, superoxide dismutase, ascorbate oxidase and tyrosinase[50, 51].Copper exist in the body in both oxidation states oxidized Cu (II) and reduced Cu (I).The actual concern in copper complexes is coming from their potential use as antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors or chemical nucleases [52].

The recommended daily intake of copper in healthy adults is between 1.5 to 3.0 mg/day[53]. Copper requires tightly regulated homeostatic mechanisms to guarantee the sufficient supplies without any toxic impacts [54]. Overload or deficiency of copper is correlated with Wilson disease (WD) and Menkes disease (MD) respectively of which both are considered of genetic origin[55].Researches on Wilson and Menkes disorders supplied helpful insights in the domain of copper homeostasis especially into understanding of intracellular trafficking and distribution of copper at molecular level, identifying potential new function for copper and copper metabolism proteins in cellular signaling, gene expression, and cancer cells

proliferation[56].In cancer cell proliferation the abnormal accumulation of copper by cancer cells might demonstrate to be a differentiating characteristic of mutated vs. healthy cells that can be targeted by novel chemotherapeutic agents. Elevated levels of copper have been found in many types of human cancers, including prostate, breast, colon, lung and brain[57]. Furthermore, a specific amount of local copper appears to be required for angiogenesis to occur[58].

At molecular level copper complexes interact directly with proteins and DNA, leading to dysfunction and cleavage of macromolecular structure, or indirectly producing reactive oxygen species that attack and degrade biomolecules[59, 60]. Besides DNA binding, intercalation and cleavage activity, superoxide dismutase mimetic activity, as well generation of ROS (reactive oxygen species) all these intra cellular events trigger cancer cell death by apoptotic mechanism. On the other hand, a non-apoptotic form of programmed cell death has been evidenced recently in human cancer cells treated with both copper (I) and copper (II) complexes[59, 61-65]. The effect of ligand chelation may also be important for biological activity of the complexes by influencing the hydrophilic/lipophilic nature of the copper complex; also their role in the stability of copper coordination compound. Regarding this, it looks that cytotoxic copper-based species should be stable enough to carry the metal to the desire target without irreversible and nonselective interactions with other biomolecules on way

to the cell, but sufficiently labile to allow the metal to interact with the appropriate binding site when the target substrate is reached[29].

The first evidence that copper complexes may have antitumor activity is as thiosemicarbazones complexes (TSCs) which is a class of medicinal compounds whose their anticancer activity has been reported in early 1960sand some of them are in clinical practice[66-68].TSCs have been heavily studied due to their inhibitory action on the DNA enzyme ribonucleotide diphosphate reductase and their selectivity to hormone-responsive cancers[69].

1.8 Copper polyamines complexes

Seven novel copper (II) complexes, derived from dipropylenetriaminetripodal N,N,N-ligand and diamines N,N-ligands, have been synthesized by Dr. Ismail Warrad team. Diverse types of diamines and triamine ligands were complexed with mononuclear dicationic Cu (II) ions in facile one pot reaction with high yield. These complexes were then characterized by spectroscopic, thermal, electrochemical and X-ray crystallographic techniques. The UV-Visible and single-crystal X-ray diffraction data show distorted trigonal bipyramidal geometry around Cu (II) ions with one solvated water molecule. The thermogravimetric analyses indicate that these complexes contain one water molecule and they decompose in three steps. The cyclic voltammetric studies indicate Cu-complex to be a quasi-reversible one-electron couple.Promising

antibacterial results were collected by testing these complexes against several bacterial families[70].

1.9 The aim of the study

Studying the anticancer activity of seven novel copper (II) polyamine complexes by assessing their in *vitro cytotoxic* and *cytostatic* activity by using cancer cell lines.

The following four cell lines were used: human muscle cell as normal cell control (L6), human prostate cancer (PC3), human colon cancer (Hct) and human liver cancer (HepG2).

Chapter Two
Material and Methods

2. Material and Methods

2.1 Chemical material

Chemical complexes were prepared by Dr. Ismail Warad team and exist as a solid blue powder in 7 vials numbered from 1 to 7 and ready to prepare stock solution[70].

2.2 Preparation of stock solution

1.5 mg of each complex were dissolved in 30 ml of media (RPMI) supplemented with 1% non-essential amino acid, 1% l-glutamine, 1% penicillin streptomycin and 1% amphotericin B . The final concentration of 0.5 mg/ml the prepared stock solution was stored at 4ºC until use.

2.3 Cell culture

2.3.1 Cell lines

Human colon cancer cells (HCT116, ATCC number: CCL-247, human, from the epithelial tissue of the colon) , human prostate cancer cells (PC3, ATCC number: CRL-1435,human, from human prostate), human liver cancer cells (HepG2, ATCC number: HB-8065, human, from the epithelial cells of the liver) and normal muscle cells (L6, ATCC number: CRL-1458, human, from the skeletal muscle) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 1% non-essential amino acid, 1% l-glutamine, 1% penicillin streptomycin and 1% amphotericin B. All cell lines were grown in a humidified atmosphere

of 95% air, 5% CO_2 at 37ºC, the culture medium was changed at least twice a week as needed. All chemicals used were purchased from Biological Industries except for the amphotericin B and MTT reagent from SIGMA Company and cell lines were provided by Prof. Bashar Saad.

2.4 Determination of cell viability

2.4.1 MTT Assay

Viable cells have the ability to reduce the yellow colored water soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to a water insoluble purple colored formazan crystals by a functional mitochondria via succinate dehydrogenase which provide a quantitative determination of viable cells[71].

After the cells were seeded in 100 µl DMEM media in 96-well plate then treated for 24h with various concentrations of the prepared stock solution of the chemical complexes, then incubated for another 24h in 37ºC. After the take of treatment solution, 100µl of MTT (0.5 mg/ml) was added then incubated for 4 hours in 37ºC. After 4 hours the MTT solution was removed and 100µl of isopropyl alcohol and formic acid (9:1) solution was added to dissolve the formazan crystals then incubated for 15 min in the dark at room temperature. After that the Optical Density of the MTT formazan was determined at 570 nm in an enzyme linked immunosorbant assay (ELISA) reader and cell viability was defined as a percentage of absorbance of treated cells to the control.

2.4.2 Cytotoxicity Assay

Cells at 70-80% confluence were detached from culture flask by removing the culture medium then adding 0.05% trypsin- EDTA and a suspension of 100 µl (2.0×10^4 cells/well) of viable cells were seeded in a 96-well plate and incubated for 24 h at 37ºC. After the removal of media cells were treated with 100 µl stock solution serially diluted to reach concentrations of 0.5, 0.25, and 0.125 mg/ml for complexes 2, 4, 5, 6 and 7, and concentrations of 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml for complexes 1 and 3, then incubated for 24 h at 37ºC to perform MTT assay.

2.4.3 Cytostatic Assay

In order to determine the cytostatic effect of the chemical complexes, a less number of cells were seeded in each well (1.0×10^4 cells/well) and incubated for 24h at 37ºC. Then the medium were removed and cells were treated with 100µl of the chemical complexes of the same concentrations as mentioned in previous section then incubated for 24h at 37ºC to perform the MTT assay.

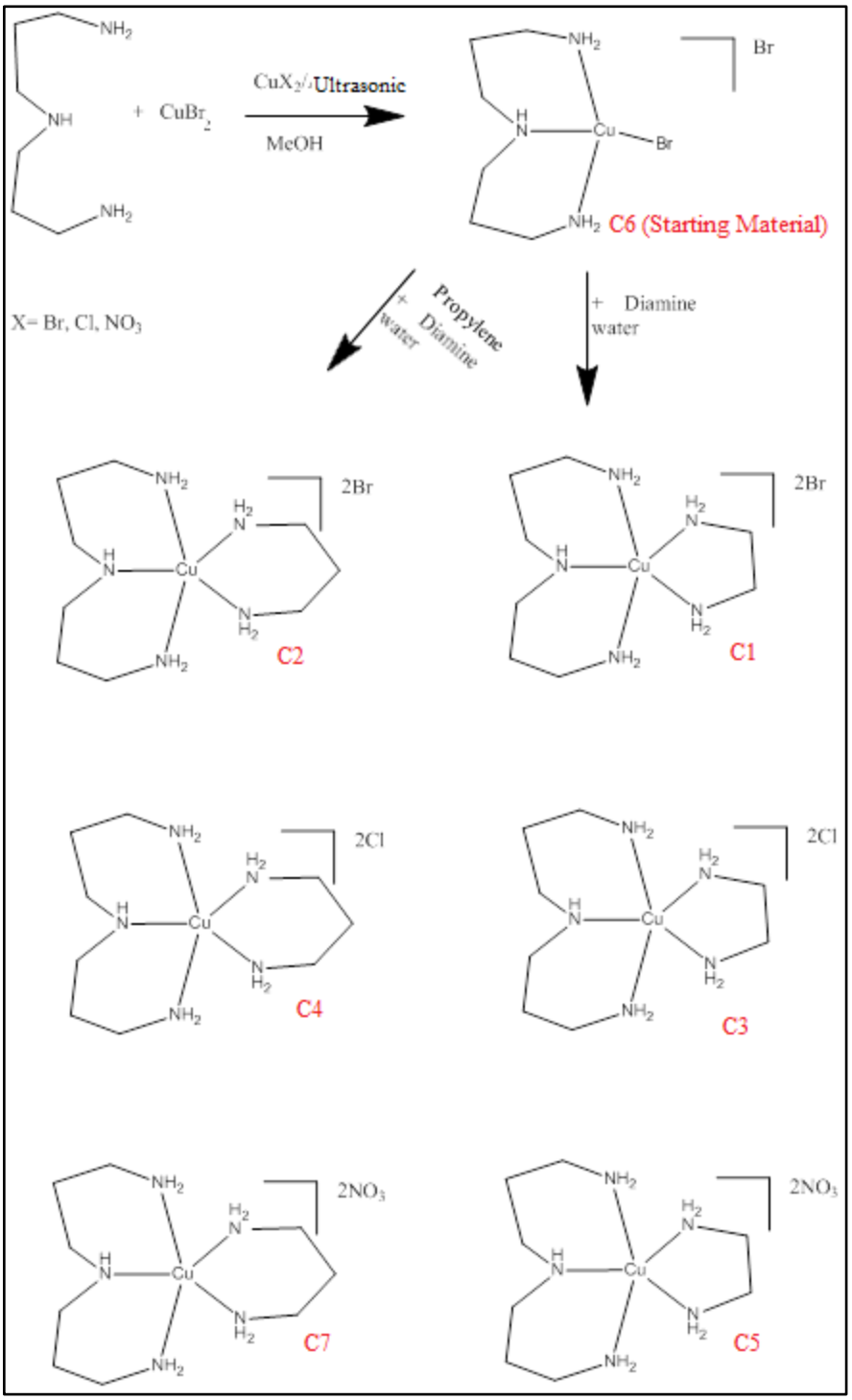
Chapter Three
Results and Discussion

3. Results and Discussion

Cancer still one of the major health issues worldwide and chemotherapy is widely used to treat cancer regardless of its side effects. We used seven novel copper (II) polyamine complexes to screen their anti-tumor effect on different cell lines, human muscle cell as normal cell control (L6), human prostate cancer (PC3), human colon cancer (Hct) and human liver cancer (HepG2) by using MTT test to evaluate the *in vitro* cytotoxic and cytostatic effects of our complexes.

3.1 Synthesis of the complexes

Mixed-ligand copper(II) complexes of the type [Cu(dipn)(N-N)] X_2 (C1–C7), dipn is dipropylenetriamine (dipn) and (N-N) is ethylenediamine (C1) and propylamine (C2), were synthesized by the reaction of amines ligands with Cu(II) ions in [1:1:1] molar ratio in a mixture of ethanol: water (Scheme 1.1). These complexes have been isolated as bromide C1-C2 or chloride C3-C4 or nitrate C5-C7 salts in good yields[70]. They have been characterized using elemental analysis and spectral methods[72] .Theses complexes are blue in color and are soluble in water.



Scheme 3.1: Synthesis of copper complexes (C1, C2, C3, C4, C5, C6 "the starting material" and C7) using different counter ions (Br, Cl and NO_3).

3.2 Cytotoxic effect of copper (II) polyamine complexes

Cells were seeded on 96 well plate, 2×10^4cell/well with 40-50% confluence, and left for 24 hours.Cells were treated with 100 µl stock solution serially diluted to reach concentrations of 0.5, 0.25, and 0.125 mg/ml for complexes 2, 4, 5, 6 and 7 respectively, and concentrations of 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml for complexes 1 and 3 then incubated for 24 hours. Cell viability was determined using MTT assay by adding MTT solution and incubated for four hours, after that isopropyl alcohol was added for 15 minutes in dark. Then absorbance was measured by using ELISA reader at 570 nm.

3.2.1 Cytotoxic effect of the complexes on L6 Cells (normal cells)

Results show that most of the complexes have a cytotoxic effect on L6 cells at concentrations higher than 0.25 mg/ml except for C6 which did not reduce the cell viability at any concentration and C1 and C3 induced cytotoxic effect at very low concentration 0.03125 mg/ml.IC50 for the complexes were C1 0.07, C2 0.18, C3 0.07, C4 0.17, C5 0.11 and C7 0.09 (Figure 3.1, Table 3.1).The lines represent the mean of three independent experiments carried out in triplicates. In fact C1 and C3 have exactly the same effect on L6 cells and that’s why their lines overlap.

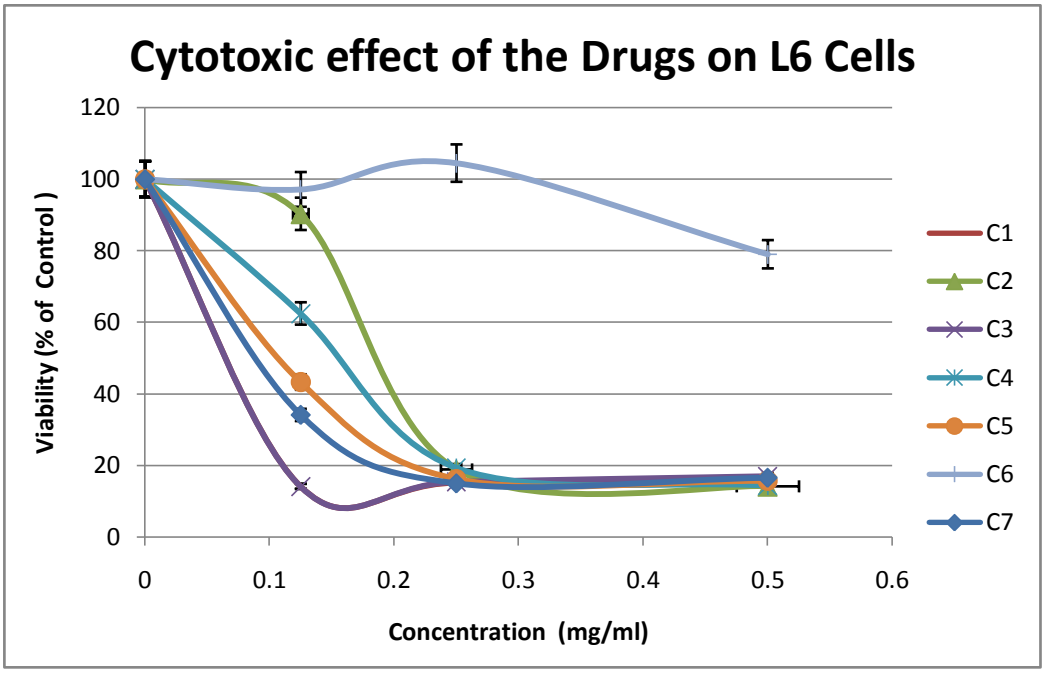


Figure 3.1: Cytotoxic experiment with L6 cellsusing different types of copper complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours, the lines represent the mean of three independent experiments carried out in triplicates**.**

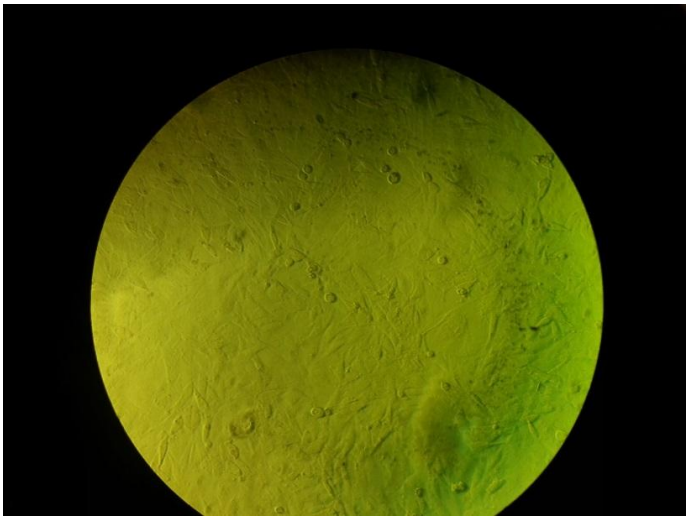


Figure 3.2: L6 cells in culture plate before seeding on 96 well plate (400X).

3.2.2 Cytotoxic effect of the complexes on Hct Cells (colon cancer)

Three complexes induced a cytotoxic effect on Hct cells at concentrations higher than 0.5 mg/ml C2, C4 and C5,while C1 and C3 induced cytotoxicity at very low concentration 0.03125 mg/ml and C7 induced cytotoxicity at concentrations higher than 0.25 mg/ml but C6 didn't reduce the cell viability at any concentration. IC50 for the complexes wereC1 0.03, C2 0.3, C3 0.015, C4 0.33, C5 0.2 and C7 0.2 (Figure 3.3, Table 3.1). The lines represent the mean of three independent experiments carried out in triplicates. Actually C1 and C3 exhibited promising antiproliferative activity against the tested cell line with IC50 values of (0.03, 0.015) These values are promising if compared with the IC50 value of the same complexes on the normal cell line L6 with value of 0.07 which indicates that these complexes have an anticancer effect at nontoxic concentrations.

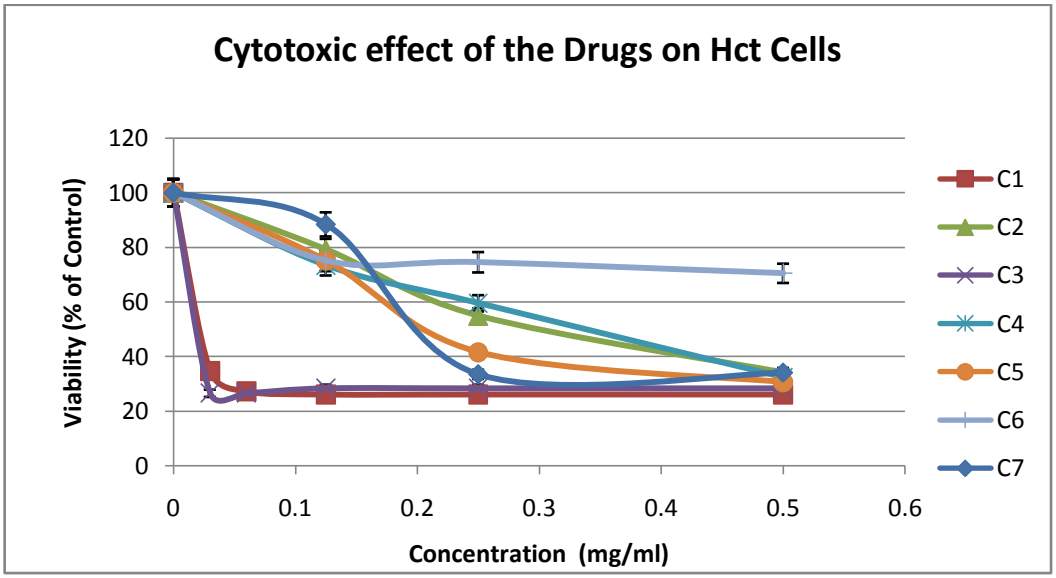


Figure 3.3: Cytotoxic experiment with Hct cells using different types of copper complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours,the lines represent the mean of three independent experiments carried out in triplicates.

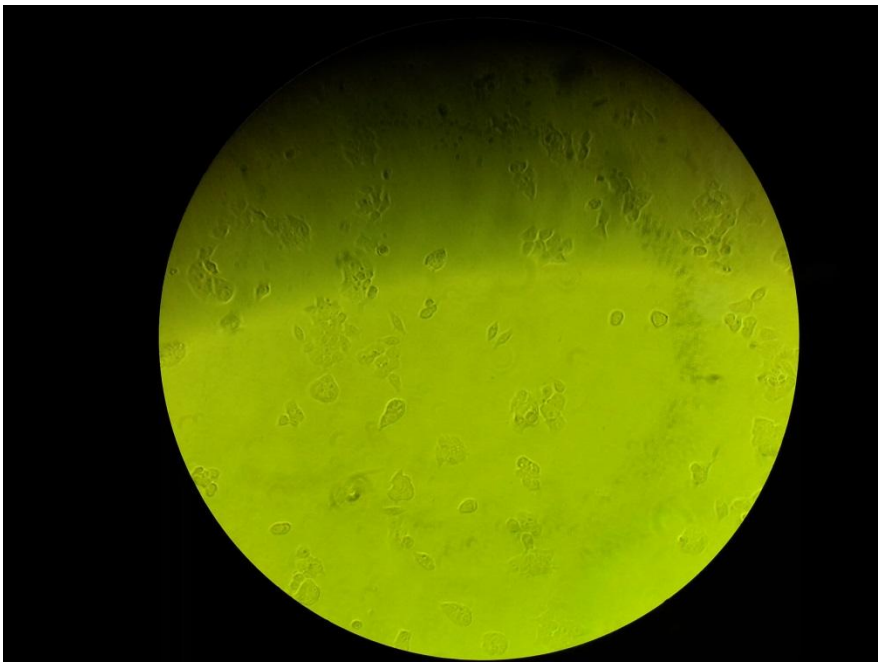


Figure 3.4: Hct cells in culture plate before seeding on 96 well plate (400X).

3.2.3 Cytotoxic effect of the complexes on PC3 cells (prostate cancer)

Four complexes induced cytotoxic effects at concentrations higher than 0.5 mg/ml on PC3 cells C2, C4, C5 and C7, while C1and C3 induced cytotoxicity at very low concentration 0.03125 mg/ml but C6 didn't reduce the cell viability at any concentration. IC50 for the complexes were C1 0.02, C2 0.17, C3 0.015, C4 0.17, C5 0.1 and C7 0.17 (Figure 3.5, Table 3.1). The lines represent the mean of three independent experiments carried out in triplicates. C1 and C3 possess promising antiproliferative activity against PC3 cell line with IC50 values of (0.02, 0.015) These values are promising if compared with the IC50 value of the same complexes with IC50 values on the normal cell line L6 with value of 0.07 which indicates that these complexes have an anticancer effect at nontoxic concentrations.

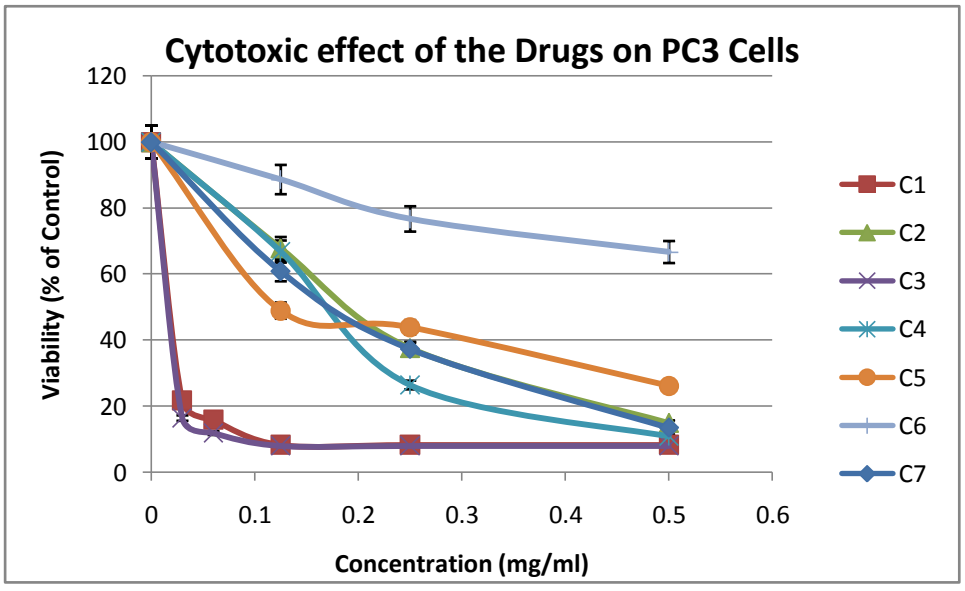


Figure 3.5: Cytotoxic experiment with PC3 cells using different types of copper complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours,the lines represent the mean of three independent experiments carried out in triplicates.

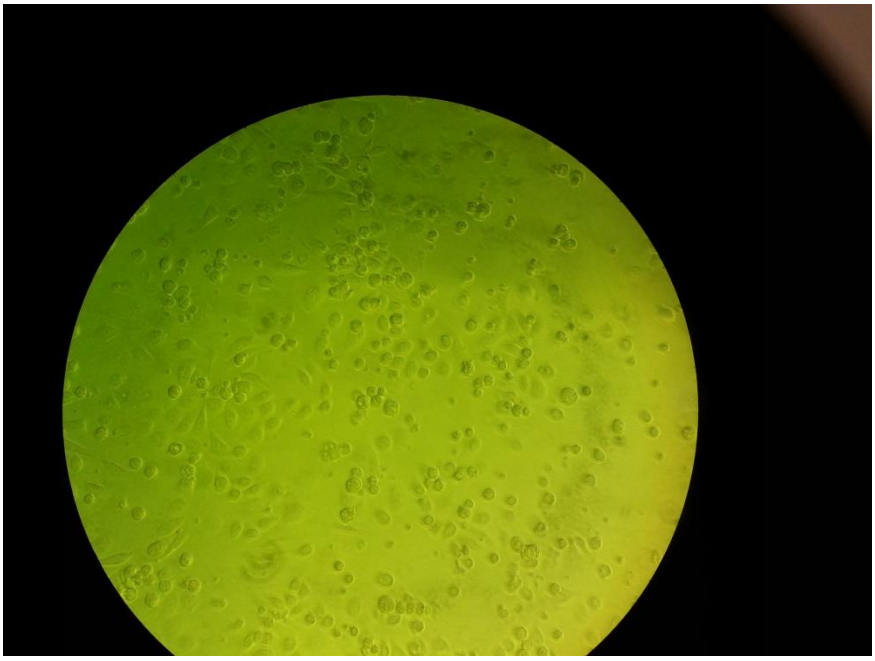


Figure 3.6: PC3 cells in culture plate before seeding on 96 well plate (400X).

3.2.4 Cytotoxic effect of the complexes on HepG2 Cells (liver cancer)

Three complexes induced cytotoxic effect at concentrations higher than 0.25 mg/ml which are C2, C4 and C5 while C1and C3 induced cytotoxic effect at very low concentration 0.03125 mg/ml. C7 induced cytotoxic effect at concentrations higher than 0.17 mg/ml but C6 didn't reduce cell viability at any concentration. IC50 for the complexes were C1 0.015, C2 0.1, C3 0.015, C4 0.18, C5 0.1 and C7 0.07(Figure 3.7, Table 3.1). The lines represent the mean of three independent experiments carried out in triplicates. Complexes C1, C3 and C2, C5 have exactly the same effect on HepG2 cells and that's why their lines overlap. C1, C3 and C7have promising antiproliferative activity against HepG2 cell line with IC50 values of (0.015, 0.015, 0.07) These values are promising if compared with

the IC50 value of the same complexes on the normal cell line L6 with value of 0.07 for C1 and C3 and 0.09 for C7 which indicates that these complexes have an anticancer effect at nontoxic concentrations.

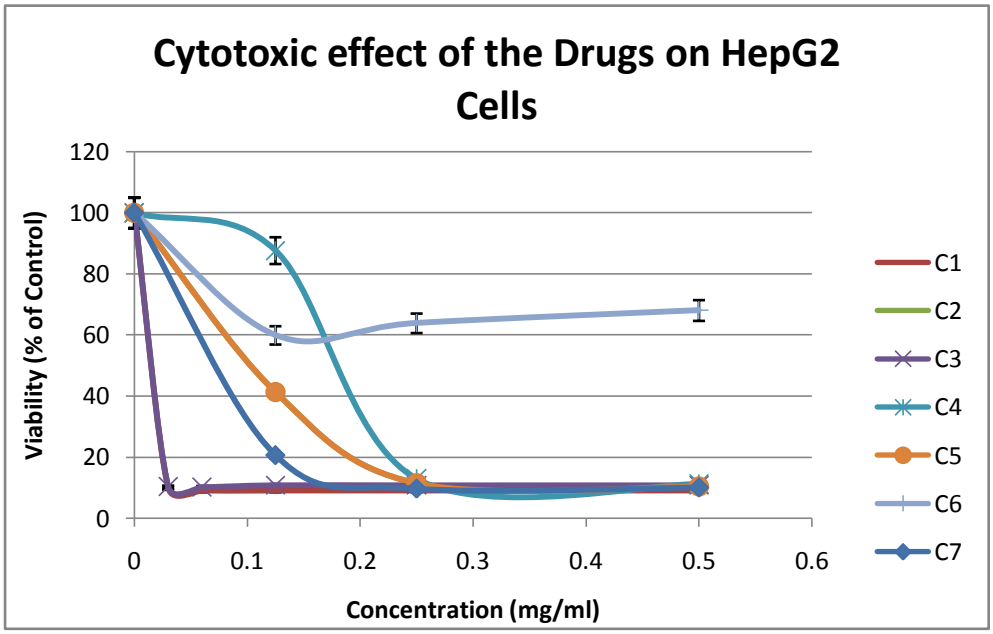


Figure 3.7: Cytotoxic experiment with HepG2 cells using different types of copper complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours,the lines represent the mean of three independent experiments carried out in triplicates.

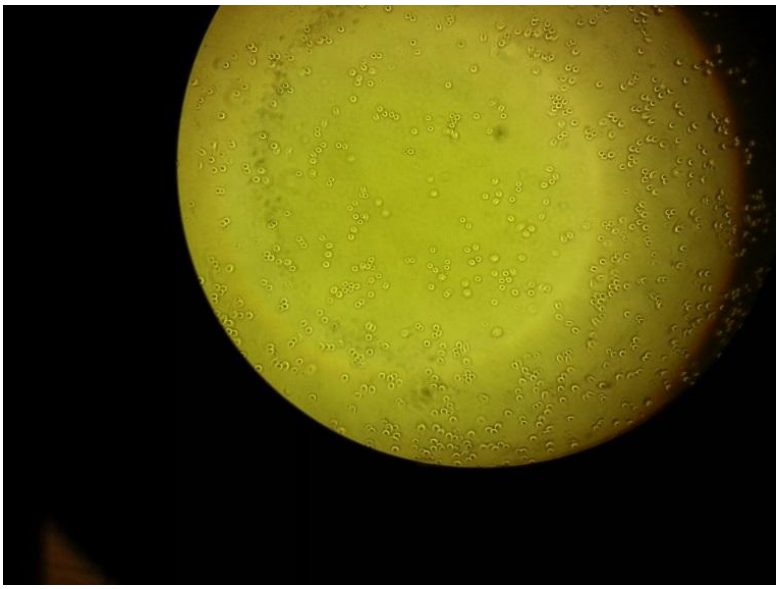


Figure 3.8: HepG2 cells in culture plate before seeding on 96 well plate (400X).

Table 3.1 IC50 for the cytotoxic effect of the complexes on L6, Hct, PC3 and HepG2.

Cell line	IC50 (mg/ml)						
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
L6	0.07±0.01	0.18±0.03	0.07±0.01	0.17±0.07	0.11±0.002	-----	0.09±0.001
Hct	0.03±0.01	0.3±0.01	0.015±0.003	0.33±0.05	0.2±0.01	-----	0.2±0.01
PC3	0.02±0.01	0.17±0.02	0.015±0.002	0.17±0.06	0.12±0.02	-----	0.17±0.05
HepG2	0.015±0.003	0.1±0.02	0.015±0.002	0.18±0.01	0.1±0.01	-----	0.07±0.01

Table 3.1:Results shows that complex 1 and complex 3 induced cytotoxic effect on Hct (IC50: 0.03, 0.015), PC3 (IC50: 0.02, 0.015) and HepG2 (IC50: 0.015, 0.015) cell lines at nontoxic level compared to its effect on L6 cell line (IC50: 0.07). Also complex 7 induced cytotoxic effect on HepG2 cell line (IC50: 0.07) at nontoxic level compared to its effect on L6 cell line (IC50: 0.09).

3.3 Cytostatic effect of copper (II) polyamine complexes

Cells were seeded on 96 well plate, 1×10^4cell/well with 40-50% confluence, and left for 24 hours. Cells were treated with 100 µl stock solution serially diluted to reach concentrations of 0.5, 0.25, and 0.125 mg/ml for complexes 2, 4, 5, 6 and 7, and concentrations of 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml for complexes 1 and 3 then incubated for 24 hours. Cell viability was determined using MTT assay by adding MTT solution and incubated for four hours, after that isopropyl alcohol was added for 15 minutes in dark. Then absorbance was measured by using ELISA reader at 570 nm.

3.3.1 Cytostatic effect of the complexes on L6 cells (normal cells)

Results shows that C1and C3 induced cytostatic effect at very low concentration 0.03125 mg/ml, C4 and C2 at higher than 0.25 mg/ml, C7 at higher than 0.125 mg/ml, C2 at higher than 0.5 mg/ml and C6 did not reduce cell viability at any concentration. IC50 of the complexes were C1 0.02, C2 0.25, C3 0.02, C4 0.18, C5 0.11, C6 0.4 and C7 0.07 (Figure 3.9, Table 3.2). The lines represent the mean of three independent experiments carried out in triplicates.

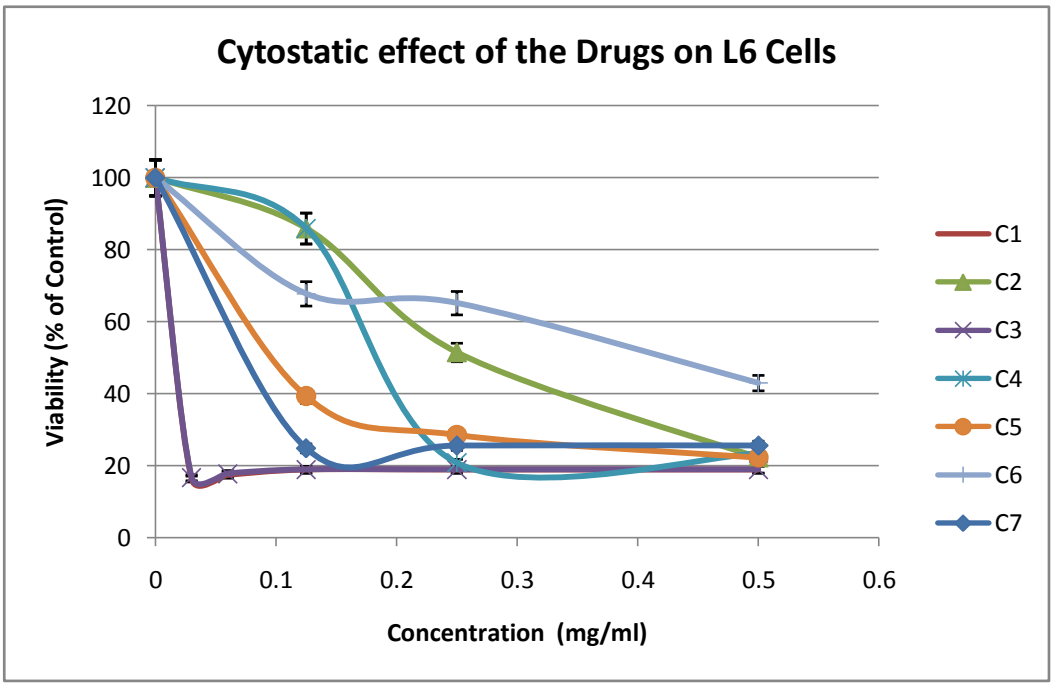


Figure 3.9: Cytostatic experiment with L6 cells using different types of copper complexes in a humidified atmosphere,cells incubated with the complexes for 24 hours,the lines represent the mean of three independent experiments carried out in triplicates.

3.3.2 Cytostatic effect of the complexes on Hct cells (colon cancer)

Four complexes induced cytostatic effect at concentrations more than 0.5 mg/ml which are C2, C4, C5 and C7. C1 induced cytostatic effect at 0.125mg/ml, C3 at very low concentration 0.03125 mg/ml and C6 didn't reduce the cell viability at any concentration. IC50 of the complexes were C1 0.04, C2 0.37, C3 0.02, C4 0.25, C5 0.27 and C7 0.18 (Figure 3.10, Table 3.2). The lines represent the mean of three independent experiments carried out in triplicates. By comparing the IC50 values of all complexes onHct cells with the IC50 of the same complexes on L6 it indicates that their cytostatic activity on Hct cells considered toxic to the normal cells and not promising.

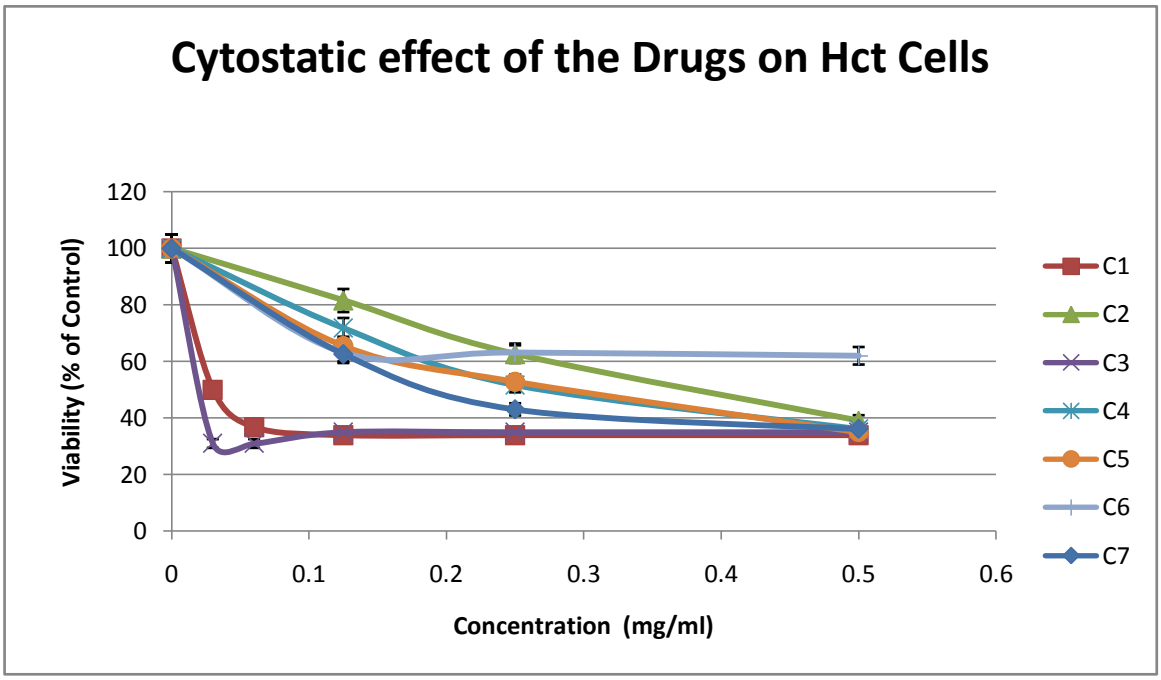


Figure 3.10: Cytostatic experiment with Hct cells using different types of copper complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours,the lines represent the mean of three independent experiments carried out in triplicates.

3.3.3 Cytostatic effect of the complexes on PC3 cells (prostate cancer)

Complexes C2, C4, C5 and C7 induced cytostatic effect at concentrations more than 0.5 mg/ml while C1 at 0.125mg/ml and C3 at very low concentration 0.03125 mg/ml and C6 didn't reduce the cell viability at any concentration. IC50 of the complexes were C1 0.015, C2 0.37, C3 0.015, C4 0.45, C5 0.17 and C7 0.19 (Figure 3.11, Table 3.2). The lines represent the mean of three independent experiments carried out in triplicates. Actually C1 and C3 exhibited promising antiproliferative activity against the tested cell line with IC50 values of 0.015 for both complexes This values are promising if compared with the IC50 value of the same complexes on the normal cell line L6 with value of 0.02 which indicates that these complexes have an anticancer effect at nontoxic concentrations.

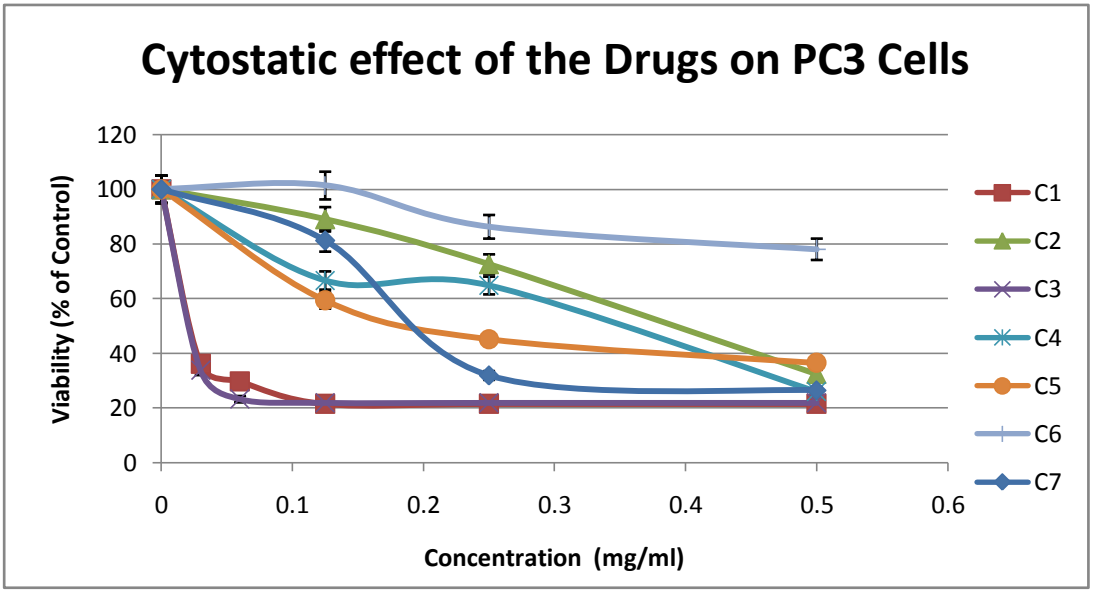


Figure 3.11: Cytostatic experiment with PC3 cells using different types of copper complexes in a humidified atmosphere,cells incubated with the complexes for 24 hours, the lines represent the mean of three independent experiments carried out in triplicates

3.3.4 Cytostatic effect of the complexes on HepG2 cells (liver cancer)

Three complexes induced cytostatic effect at 0.25 mg/ml C2, C4 and C7 while C5 at 0.17mg/ml, C1 and C3 at very low concentration 0.03125 mg/ml and C6 didn't reduce the cell viability at any concentration. IC50 of the complexes were C1 0.015, C2 0.15, C3 0.015, C4 0.13, C5 0.09 and C7 0.11(Figure 3.12, Table 3.2). The lines represent the mean of three independent experiments carried out in triplicates. C1, C3, C4 and C5 have promising antiproliferative activity against HepG2 cell line with IC50 values of (0.015, 0.015, 0.13, and 0.09). These values are promising if compared with the IC50 value of the same complexes on the normal cell line L6 with value of 0.02 for C1 and C3, 0.18 for C4 and 0.1 for C5 which indicates that these complexes have an anticancer effect at nontoxic concentrations.

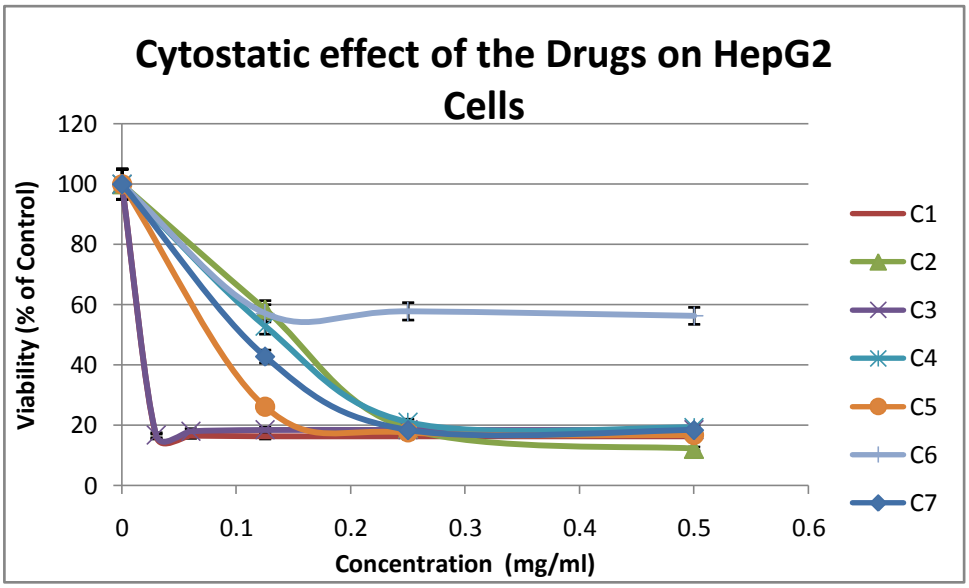


Figure 3.12: Cytostatic experiment with HepG2 cells using different types of copper complexes in a humidified atmosphere,cells incubated with the complexes for 24 hours, the lines represent the mean of three independent experiments carried out in triplicates.

Table 3.2 IC50 for the cytostatic effect of the complexes on L6, Hct, PC3 and HepG2.

Cell line	IC50 (mg/ml)						
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
L6	0.02±0.001	0.25±0.02	0.02±0.001	0.18±0.03	0.1±0.03	0.4±0.01	0.07±0.01
Hct	0.04±0.003	0.37±0.04	0.02±0.001	0.25±0.05	0.27±0.07	> 0.1-	0.18±0.03
PC3	0.015±0.002	0.37±0.04	0.015±0.002	0.35±0.03	0.17±0.06	> 0.1-	0.19±0.04
HepG2	0.015±0.002	0.15±0.002	0.015±0.002	0.13±0.01	0.09±0.01	> 0.1-	0.11±0.02

Table 3.2: Results shows that complex 1 and complex 3 induced cytostatic effect on PC3 and HepG2 (IC50: 0.015) cell lines at nontoxic level compared to its effect on L6 cell line (IC50: 0.02), also complex 4 and complex 5 induced cytostatic effect on HepG2 cell line (IC50: 0.13, 0.09) at nontoxic level compared to its effect on L6 cell line (IC50: 0.18, 0.1).

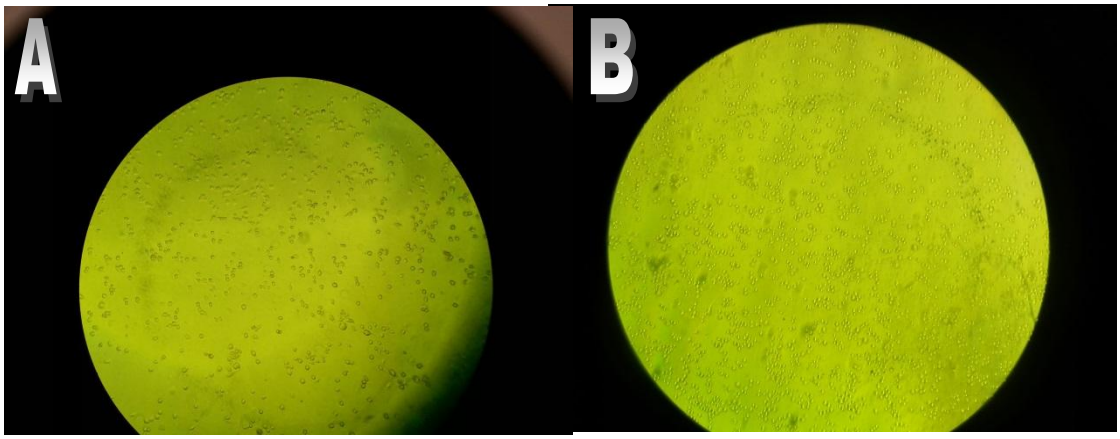


Figure 3.13: (A) HepG2 cells before treatment (Photo taken at 400X). **(B)** HepG2 cells after treatment with 0.125mg/ml of C1 (Photo taken at 400X).

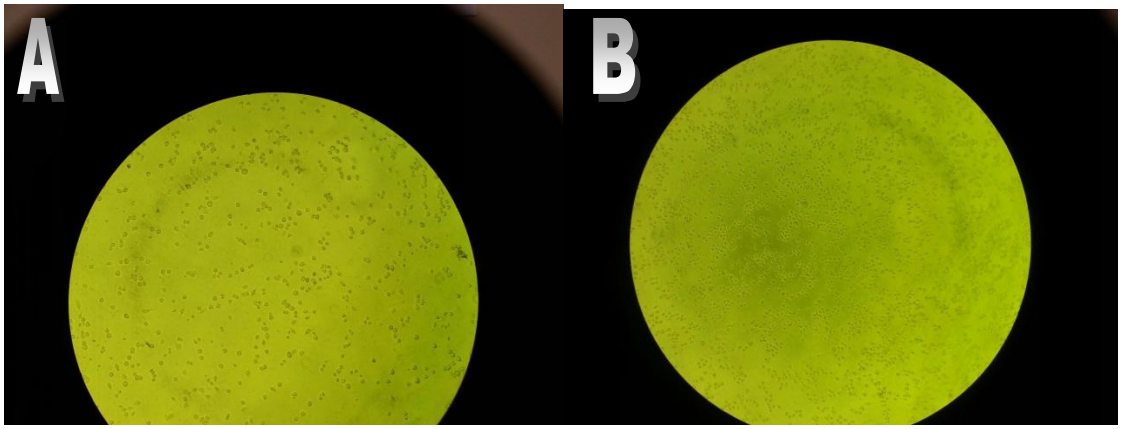


Figure 3.14: (A) HepG2 cells before treatment (Photo taken at 400X). **(B)** HepG2 cells after treatment with 0.125mg/ml of C3 (Photo taken at 400X).

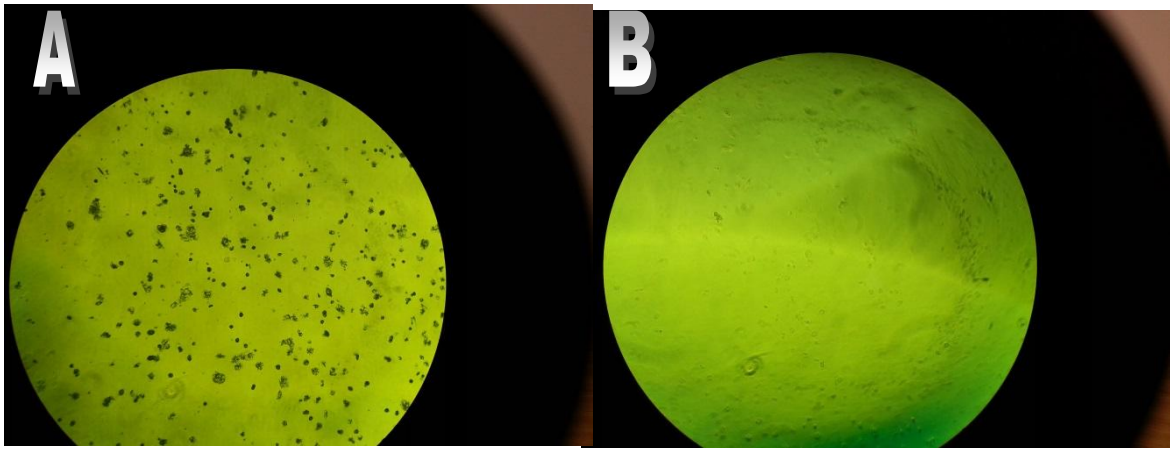


Figure 3.15: (A) HepG2 cells after 4 hours incubation with MTT formazan crystals are clearly visible (Photo taken at 400X). **(B)** HepG2 cells after 15 minute incubation with isopropanol in dark formazan crystals are dissolved (Photo taken at 400X).

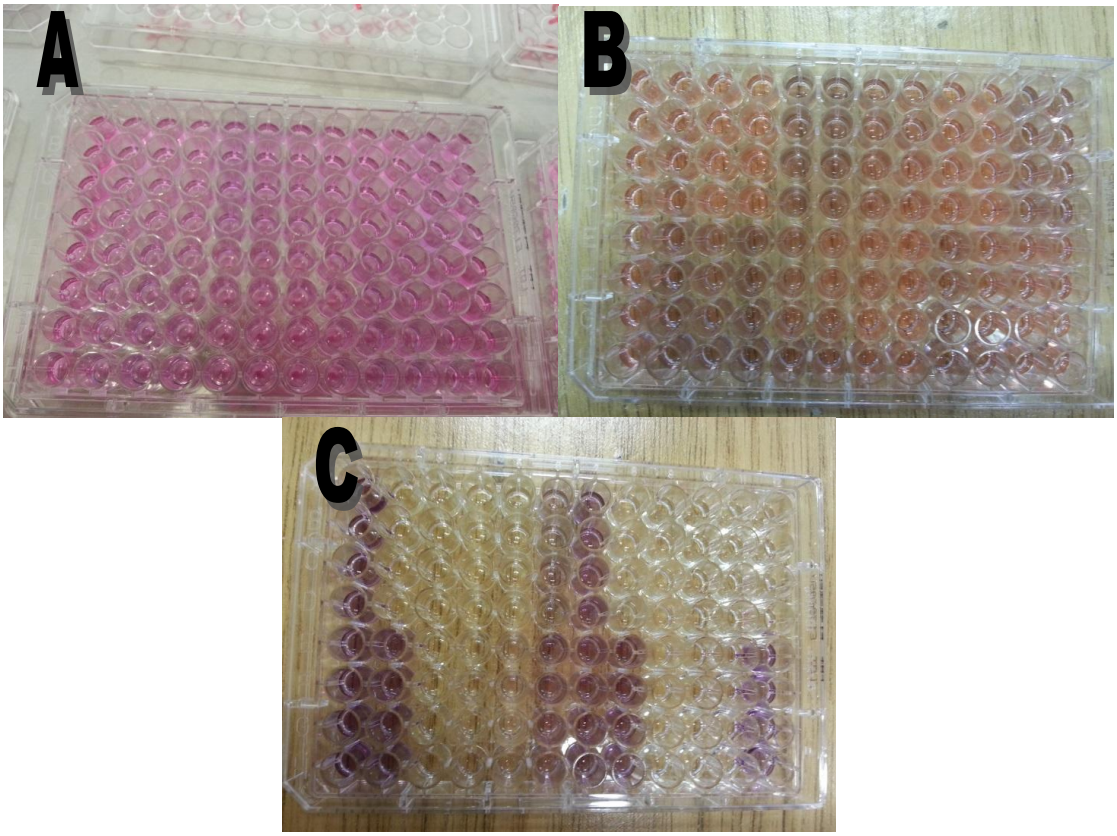


Figure 3.16: (A) 96 well plate after treatment with different concentration of complexes C1, C2, C3, C4, C5, C6 and C7. **(B)** The same 96 well plate after 4 hours incubation with MTT. **(C)** The same 96 well plate after 15 minute incubation in dark with isopropanol.

Conclusion and Perspective

In conclusion, such of these complexes revealed promising results as anti-proliferative agents in nontoxic levels on different cancer cell lines for example C1 and C3 which induced cytotoxic effect and cytostatic effect at very low concentrations so in further studies we recommend to use even lower concentration in order to decrease the toxicity level to its minimum. We also recommended doing additional studies to determine apoptosis such as caspase-3, DNA fragmentation and DNA laddering.

References

1. Bray, F., A. Jemal, N. Grey, J. Ferlay, and D. Forman, **Global cancer transitions according to the Human Development Index (2008?2030): a population-based study.** The Lancet Oncology, 2012. 13(8): p. 790-801.

2. Ferlay, J., H.R. Shin, F. Bray, D. Forman, C. Mathers, and D.M. Parkin, **Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.** Int J Cancer, 2010. 127(12): p. 2893-917.

3. Sakarkar, D. and V. Deshmukh, ***Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity.*** **International Research Journal of PharmTech Research, 2011. 3(1): p. 298-308.**

4. Sharma, H., L. Parihar, and P. Parihar, **Review on cancer and anticancerous properties of some medicinal plants.** J Med Plants Res, 2011. 5(10): p. 1818-1835.

5. Anand, P., A.B. Kunnumakara, C. Sundaram, K.B. Harikumar, S.T. Tharakan, O.S. Lai, B. Sung, and B.B. Aggarwal, **Cancer is a preventable disease that requires major lifestyle changes.** Pharmaceutical research, 2008. 25(9): p. 2097-2116.

6. Evan, G.I. and K.H. Vousden, **Proliferation, cell cycle and apoptosis in cancer.** Nature, 2001. 411(6835): p. 342-348.

7. S. Kameshwaran, V.Suresh, G. Arunachalam, S.K.Kanthlal, and M.Mohanraj, ***In Vitro and In Vivo Anti Cancer Activity Of Methanolic***

Extract Of Tecoma Stans Flowers. **International Research Journal of Pharmacy,** 2012. 3(3): p. 246-251.

8. Bouwman, P. and J. Jonkers, **The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance.** Nat Rev Cancer, 2012. 12(9): p. 587-98.

9. Pisani, P., F. Bray, and D.M. Parkin, ***Estimates of the world-wide prevalence of cancer for 25 sites in the adult population.*** **International Journal of Cancer, 2002. 97(1): p. 72-81.**

10. Bosch, F.X., J. Ribes, R. Cléries, and M. Díaz, **Epidemiology of hepatocellular carcinoma.** Clinics in liver disease, 2005. 9(2): p. 191-211.

11. Hertl, M. and A.B. Cosimi, **Liver Transplantation for Malignancy.** The Oncologist, 2005. 10(4): p. 269-281.

12. Olsen, S.K., R.S. Brown, and A.B. Siegel, ***R*eview: Hepatocellular carcinoma: review of current treatment with a focus on targeted molecular therapies.** Therapeutic advances in gastroenterology, 2010. 3(1): p. 55-66.

13. Tinkle, C.L. and D. Haas-Kogan, **Hepatocellular carcinoma: natural history, current management, and emerging tools.** Biologics: targets & therapy, 2012. 6: p. 207.

14. Schoen, R.E., **The case for population-based screening for colorectal cancer.** Nature Reviews Cancer, 2002. 2(1): p. 65-70.

15. Bond, J.H., ***Polyp guideline: diagnosis, treatment, and surveillance for patients with colorectal polyps.*** **The American journal of gastroenterology,** 2000. 95(11): p. 3053-3063.

16. Terdiman, J.P., P.G. Conrad, and M.H. Sleisenger, ***Genetic testing in hereditary colorectal cancer: indications and procedures*****. The American journal of gastroenterology, 1999. 94(9): p. 2344-2356.**

17. Lamprecht, S.A. and M. Lipkin, **Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms.** Nature Reviews Cancer, 2003. 3(8): p. 601-614.

18. Chen, W.-D., Z.J. Han, J. Skoletsky, J. Olson, J. Sah, L. Myeroff, P. Platzer, S. Lu, D. Dawson, and J. Willis, ***Detection in fecal DNA of colon cancer–specific methylation of the nonexpressed vimentin gene.*** **Journal of the National Cancer Institute,** 2005. 97(15): p. 1124-1132.

19. De Marzo, A.M., E.A. Platz, S. Sutcliffe, J. Xu, H. Grönberg, C.G. Drake, Y. Nakai, W.B. Isaacs, and W.G. **Nelson, Inflammation in prostate carcinogenesis.** Nature Reviews Cancer, 2007. 7(4): p. 256-269.

20. Sutcliffe, S. and G.A. Colditz, ***Prostate cancer: is it time to expand the research focus to early-life exposures?*** Nature Reviews Cancer, 2013. 13(3): p. 208-518.

21. Jemal, A., T. Murray, E. Ward, A. Samuels, R.C. Tiwari, A. Ghafoor, E.J. Feuer, and M.J. Thun, ***Cancer statistics, 2005.*** CA: a cancer journal for clinicians, 2005. 55(1): p. 10-30.

22. Gonzalgo, M.L. and W.B. Isaacs, ***Molecular pathways to prostate cancer.*** The Journal of urology, 2003. 170(6): p. 2444-2452.

23. Shand, R.L. and E.P. Gelmann, **Molecular biology of prostate-cancer pathogenesis.** Current opinion in urology, 2006. 16(3): p. 123-131.

24. Denmeade, S.R. and J.T. Isaacs, **A history of prostate cancer treatment.** Nature Reviews Cancer, 2002. 2(5): p. 389-396.

25. Altman, A.J., F.G. Crussi, W.J. Rierden, and R.L. Baehner, *Growth of Rhabdomyosarcoma Colonies from Pleural Fluid.* Cancer Research, 1975. 35(7): p. 1809-1812.

26. Daniel, K.G., P. Gupta, R.H. Harbach, W.C. Guida, and Q.P. Dou, **Organic copper complexes as a new class of proteasome inhibitors and apoptosis inducers in human cancer cells.** Biochem Pharmacol, 2004. 67(6): p. 1139-51.

27. Luqmani, Y.A., **Mechanisms of drug resistance in cancer chemotherapy.** Med Princ Pract, 2005. 14 Suppl 1: p. 35-48.

28. Ho, Y.-P., S.C.F. Au-Yeung, and K.K.W. To, **Platinum-based anticancer agents: Innovative design strategies and biological perspectives.** Medicinal Research Reviews, 2003. 23(5): p. 633-655.

29. Tisato, F., C. Marzano, M. Porchia, M. Pellei, and C. Santini, **Copper in diseases and treatments, and copper-based anticancer strategies.** Med Res Rev, 2010. 30(4): p. 708-49.

30. Zhang, C.X. and S.J. Lippard, ***N*ew metal complexes as potential therapeutics. C**urrent Opinion in Chemical Biology, 2003. 7(4): p. 481-489.

31. Kelland, L.R., **Preclinical Perspectives on Platinum Resistance.** Drugs, 2000. 59(S4): p. 1-8.

32. Marzano, C., M. Pellei, F. Tisato, and C. Santini, ***C*opper complexes as anticancer agents.** Anticancer Agents Med Chem, 2009. 9(2): p. 185-211.

33. Ott, I. and R. Gust, **Non Platinum Metal Complexes as Anti-cancer Drugs.** Archiv der Pharmazie, 2007. 340(3): p. 117-126.

34. Bruijnincx, P.C. and P.J. Sadler, **New trends for metal complexes with anticancer activity.** Current opinion in chemical biology, 2008. 12(2): p. 197-206.

35. Orvig, C. and M.J. Abrams, **Medicinal Inorganic Chemistry: Introduction.** Chemical Reviews, 1999. 99(9): p. 2201-2204.

36. Muhammad, N. and Z. Guo, **Metal-based anticancer chemotherapeutic agents.** Current opinion in chemical biology, 2014. 19: p. 144-153.

37. Chang, C.J. and C. He**, Using chemistry to study and control metals in biology.** Current Opinion in Chemical Biology, 2013. 17(2): p. 127-128.

38. Warad, I., F.E. Ala'a, M.A. Al-Nuri, A.I. Husein, M. Assal, A. Abu-Obaid, N. Al-Zaqri, T.B. Hadda, and B. Hammouti, ***Metal ions as Antitumor Complexes-Review.***

39. Che, C.-M. and F.-M. Siu, ***Metal complexes in medicine with a focus on enzyme inhibition.*** Current Opinion in Chemical Biology, 2010. 14(2): p. 255-261.

40. Pruefer, F.G., F. Lizarraga, V. Maldonado, and J. Melendez-Zajgla, ***Participation of Omi Htra2 Serine-Protease Activity in the Apoptosis Induced by Cisplatin on SW480 Colon Cancer Cells.*** Journal of Chemotherapy, 2008. 20(3): p. 348-354.

41. Jamieson, E.R. and S.J. Lippard, ***Structure, recognition, and processing of cisplatin-DNA adducts.*** Chemical reviews, 1999. 99(9): p. 2467-2498.

42. Lee, K.-B., D. Wang, S.J. Lippard, and P.A. Sharp, ***Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II in vitro.*** Proceedings of the National Academy of Sciences, 2002. 99(7): p. 4239-4244.

43. Jakupec, M.A., M. Galanski, V.B. Arion, C.G. Hartinger, and B.K. Keppler, ***Antitumour metal compounds: more than theme and variations.*** Dalton transactions, 2008(2): p. 183-194.

44. Yang, P. and M. Guo, ***Interactions of organometallic anticancer agents with nucleotides and DNA.*** Coordination chemistry reviews, 1999. 185: p. 189-211.

45. Belicchi Ferrari, M., F. Bisceglie, G. Pelosi, P. Tarasconi, R. Albertini, A. Bonati, P. Lunghi, and S. Pinelli, ***Synthesis, characterisation, X-ray structure and biological activity of three new 5-formyluracil thiosemicarbazone complexes.*** Journal of inorganic biochemistry, 2001. 83(2): p. 169-179.

46. Belicchi-Ferrari, M., F. Bisceglie, A. Buschini, S. Franzoni, G. Pelosi, S. Pinelli, P. Tarasconi, and M. Tavone, ***Synthesis, structural characterization and antiproliferative and toxic bio-activities of copper(II) and nickel(II) citronellal N4-ethylmorpholine thiosemicarbazonates.*** J Inorg Biochem, 2010. 104(2): p. 199-206.

47. Ferrari, M.B., F. Bisceglie, G.G. Fava, G. Pelosi, P. Tarasconi, R. Albertini, and S. Pinelli, ***Synthesis, characterization and biological activity of two new polymeric copper (II) complexes with α-ketoglutaric acid thiosemicarbazone.*** Journal of inorganic biochemistry, 2002. 89(1-2): p. 36-44.

48. Rodrıguez-Argüelles, M.C., M.B. Ferrari, F. Bisceglie, C. Pelizzi, G. Pelosi, S. Pinelli, and M. Sassi***, Synthesis, characterization and biological activity of Ni, Cu and Zn complexes of isatin hydrazones.*** Journal of inorganic biochemistry, 2004. 98(2): p. 313-321.

49. Harris, Z. and J. Gitlin, ***Genetic and molecular basis for copper toxicity.*** The American journal of clinical nutrition, 1996. 63(5): p. 836S-841S.

50. Aust, S.D., L.A. Morehouse, and C.E. Thomas***, Role of metals in oxygen radical reactions.*** **J** Free Radic Biol Med, 1985. 1(1): p. 3-25.

51. Halliwell, B. and J.M. Gutteridge, ***Role of free radicals and catalytic metal ions in human disease: an overview.*** Methods Enzymol, 1990. 186: p. 1-85.

52. Weder, J.E., C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay, J.R. Biffin, H.L. Regtop, and N.M. Davies, ***Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized.*** Coordination Chemistry Reviews, 2002. 232(1–2): p. 95-126.

53. Milne, D.B., ***Copper intake and assessment of copper status.*** The American journal of clinical nutrition, 1998. 67(5): p. 1041S-1045S.

54. Linder, M.C. and M. Hazegh-Azam***, Copper biochemistry and molecular biology.*** Am J Clin Nutr, 1996. 63(5): p. 797S-811S.

55. Bull, P.C. and D.W. Cox, ***Wilson disease and Menkes disease: new handles on heavy-metal transport.*** **Trends in genetics:** TIG, 1994. 10(7): p. 246.

56. Turski, M.L. and D.J. Thiele, ***New roles for copper metabolism in cell proliferation, signaling, and disease.*** Journal of Biological Chemistry, 2009. 284(2): p. 717-721.

57. Daniel, K.G., R.H. Harbach, W.C. Guida, and Q.P. Dou, ***Copper storage diseases: Menkes, Wilsons, and cancer.*** Front Biosci, 2004. 9: p. 2652-2662.

58. Goodman, V., G. Brewer, and S. Merajver***, Copper deficiency as an anti-cancer strategy.*** Endocrine-related cancer, 2004. 11(2): p. 255-263.

59. Marzano, C., M. Pellei, S. Alidori, A. Brossa, G.G. Lobbia, F. Tisato, and C. Santini, ***New copper (I) phosphane complexes of dihydridobis (3-nitro-1, 2, 4-triazolyl) borate ligand showing cytotoxic activity.*** **Journal of inorganic biochemistry, 2006. 100(2): p. 299-304.**

60. Iakovidis, I., I. Delimaris, and S.M. Piperakis, ***Copper and its complexes in medicine: a biochemical approach.*** Mol Biol Int, 2011. 2011: p. 594529.

61. Dallavalle, F., F. Gaccioli, R. Franchi-Gazzola, M. Lanfranchi, L. Marchiò, M.A. Pellinghelli, and M. Tegoni, ***Synthesis, molecular structure, solution equilibrium, and antiproliferative activity of***

thioxotriazoline and thioxotriazole complexes of copper (II) and palladium (II). Journal of Inorganic Biochemistry, 2002. 92(2): p. 95-104.

62. Tardito, S., O. Bussolati, F. Gaccioli, R. Gatti, S. Guizzardi, J. Uggeri, L. Marchio, M. Lanfranchi, and R. Franchi-Gazzola**,** ***Non-apoptotic programmed cell death induced by a copper (II) complex in human fibrosarcoma cells.*** Histochemistry and cell biology, 2006. 126(4): p. 473-482.

63. Tardito, S., O. Bussolati, M. Maffini, M. Tegoni, M. Giannetto, V. Dall'Asta, R. Franchi-Gazzola, M. Lanfranchi, M.A. Pellinghelli, and C. Mucchino, ***Thioamido coordination in a thioxo-1, 2, 4-triazole copper (II) complex enhances nonapoptotic programmed cell death associated with copper accumulation and oxidative stress in human cancer cells.*** Journal of medicinal chemistry, 2007. 50(8): p. 1916-1924.

64. Marzano, C., M. Pellei, D. Colavito, S. Alidori, G.G. Lobbia, V. Gandin, F. Tisato, and C. **Santini,** ***Synthesis, characterization, and in vitro antitumor properties of tris (hydroxymethyl) phosphine copper (I) complexes containing the new bis (1, 2, 4-triazol-1-yl) acetate ligand.*** Journal of medicinal chemistry, 2006. 49(25): p. 7317-7324.

65. Marzano, C., V. Gandin, M. Pellei, D. Colavito, G. Papini, G.G. Lobbia, E. Del Giudice, M. Porchia, F. Tisato, and C. **Santini,** ***In vitro antitumor activity of the water soluble copper (I) complexes bearing the***

tris (hydroxymethyl) phosphine ligand. Journal of medicinal chemistry, 2008. 51(4): p. 798-808.

66. Crim, J.A. and H.G. Petering, ***The antitumor activity of Cu (II) KTS, the copper (II) chelate of 3-ethoxy-2-oxobutyraldehyde bis (thiosemicarbazone).*** Cancer Research, 1967. 27(7): p. 1278-1285.

67. Taylor, M.R., E.J. Gabe, J.P. Glusker, J.A. Minkin, and A. Patterson, ***The Crystal Structures of Compounds with Antitumor Activity. 2-Keto-3-ethoxybutyraldehyde Bis (thiosemicarbazone) and Its Cupric Complex1.*** Journal of the American Chemical Society, 1966. 88(8): p. 1845-1846.

68. Feun, L., M. Modiano, K. Lee, J. Mao, A. Marini, N. Savaraj, P. Plezia, B. Almassian, E. Colacino, and J. Fischer, ***Phase I and pharmacokinetic study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) using a single intravenous dose schedule.*** Cancer chemotherapy and pharmacology, 2002. 50(3): p. 223-229.

69. Murugkar, A., B. Unnikrishnan, S. Padhye, R. Bhonde, S. Teat, E. Triantafillou, and E. Sinn, ***Hormone anchored metal complexes. 1. Synthesis, structure, spectroscopy and in vitro antitumor activity of testosterone acetate thiosemicarbazone and its metal complexes.*** Metal-Based Drugs, 1999. 6(3): p. 177.

70. M, A.L.-N., M.I. Choudhary, F.F. Awwadi, W.H. Talib, T.B. Hadda, S. Yousuf, A. Sawafta, and I. Warad, ***Characterization and biological activities of two copper(II) complexes with dipropylenetriamine and***

diamine as ligands. Spectrochim Acta A Mol Biomol Spectrosc, 2014. 127: p. 225-30.

71. Sieuwerts, A.M., J.G. Klijn, H.A. Peters, and J.A. Foekens, ***The MTT Tetrazolium Salt Assay Scrutinized: How to Use this Assay Reliably to Measure Metabolic Activity of Cell Cultures in vitro for the Assessment of Growth Characteristics, IC50-Values and Cell Survival.*** Clinical Chemistry and Laboratory Medicine, 1995. 33(11): p. 813-824.

72. Addison, A.W., T.N. Rao, J. Reedijk, J. van Rijn, and G.C. Verschoor, ***Synthesis, structure, and spectroscopic properties of copper (II) compounds containing nitrogen–sulphur donor ligands; the crystal and molecular structure of aqua [1, 7-bis (N-methylbenzimidazol-2'-yl)-2, 6-dithiaheptane] copper (II) perchlorate.*** J. Chem. Soc., Dalton Trans., 1984(7): p. 1349-1356.

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التقييم المخبري لنشاط عائلة مبتكرة من معقدات النحاس المرتبطة بالأمينات المتعددة في علاج خلايا السرطان في المختبر

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس – فلسطين.

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الملخص

ان معدلات الإصابة والوفيات الناتجة عن مرض السرطان في تزايد مستمر في جميع أنحاء العالم سواء في الدول النامية والمتقدمة على حد سواء ما يجعل من السرطان مشكلة صحية عالمية كبيرة. لقد قدمت الكثير من الأبحاث والجهود من اجل علاج السرطان أو وقف انتشاره أو حتى التقليل من أضراره. يستخدم العلاج الكيميائي على نطاق واسع لعلاج السرطان ولكن تبقى أثاره الجانبية والسمية مصدر قلق كبير. يعد السيسبلاتين احد أهم الاكتشافات في العلاج الكيميائي للسرطان حيث اثبت فعاليته في القضاء على أنواع مختلفة من السرطان وخاصة سرطان الخصية حيث يتعدى معدل الشفاء 90%. ومع ذلك فان العلاج بالسيسبلاتين تحده العديد من الآثار الجانبية بالإضافة للمقاومة الموروثة والمكتسبة للعلاج. هذه العقبات وغيرها شجعت في زيادة الأبحاث عن مركبات تعتمد على معادن أخرى تكون نشطة في مقاومة الخلايا السرطانية وذات خصائص دوائية محسنة.

تبحث هذه الدراسة في المركبات المكونة من النحاس المرتبط بالامينات متعددة المنح ومدى فاعليتها في تثبيط الخلايا السرطانية. تم اختيار سبع مركبات من اجل فحص تأثيرها على السرطان وذلك بتعريض ثلاثة أنواع من الخلايا السرطانية (القولون، البروستاتا والكبد) لتراكيز متزايدة من تلك المركبات ومن ثم استخدام صبغة MTT لفحص مدى سمية هذه المركبات كما تم استخدام خلايا سليمة كمجوعة ضابطة للاختبار (خلايا عضلية هيكلية). أظهرت الدراسة

فعالية بعض المركبات في تثبيط بعض أنواع الخلايا السرطانية في مستويات غير سامة للخلايا السليمة مما يجعل البحث في هذه المركبات مبشراً.