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Biochemical Studies on Some Novel Organometallic Complexes as Anti-Human Prostate Cancer

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Abstract
This study determined the selective cytotoxicity and mutagenic potential of five Schiff base complexes nanoparticles of copper salt against prostate cancer cell line (PC-3). The electron microscope data confirmed the Nano form of these complexes. *Invitro* antitumor activity of the complexes had been studied against breast cancer cell lines and the IC_{50} values were detected. The *invivo* cytotoxicity of the complexes showed that, the complexes have no side effects after six weeks was confirmed by some clinical studies. The more effective and probable binding modes between the studied metal complexes NPs with different active sites of prostate cancer were investigated using molecular docking studies. The results augur well for prostate cancer treatment. It was found that the complex nanoparticles under study can interact with antioxidant to form cancer-specific proteasome inhibitors and apoptosis inducers in prostate cancer cell.

Keywords
Cytotoxicity, Mutagenic potential, Schiff base, PC-3 cell line, Prostate cancer

Introduction

Metals are essential cellular components selected by nature to function in several indispensable biochemical processes for living organisms [1]. Metals are endowed with unique characteristics that include redox activity, variable coordination modes, and reactivity towards organic substrates. Due to their reactivity, metals are tightly regulated under normal conditions and aberrant metal ion concentrations are associated with various pathological disorders, including cancer [2]. For these reasons, coordination complexes, either as drugs or pro-drugs, become very attractive probes as potential anticancer agents. The use of metals and their salts for medicinal purposes, from iatrochemistry to modern day, has been present throughout human history. The discovery of cisplatin, *cis*-$[Pt^{II}(NH_3)_2Cl_2]$, was a defining moment which triggered the interest in platinum (II)- and other metal-containing complexes as potential novel anticancer drugs [3]. Other interests in this field address concerns for uptake, toxicity, and resistance to metallodrugs. This review article highlights selected metals that have gained considerable interest in both the development and the treatment of cancer. For example, copper is enriched in various human cancer tissues and is a co-factor essential for tumor angiogenesis processes [4]. However the use of copper-binding ligands to target tumor copper could provide a novel strategy for cancer selective treatment. The use of nonessential metals as probes to target molecular pathways as anticancer agents is also emphasized [5]. Finally, based on the interface between molecular biology and bioinorganic chemistry the design of coordination complexes for

cancer treatment is reviewed and design strategies and mechanisms of action are discussed. Prostate cancer begins when cells in the prostate gland start to grow out of control [6]. The prostate is a gland found only in males. It makes some of the fluid that is part of semen. The prostate is below the bladder (the hollow organ where urine is stored) and in front of the rectum (the last part of the intestines) [7]. Just behind the prostate are glands called seminal vesicles that make most of the fluid for semen. The urethra, which is the tube that carries urine and semen out of the body through the penis, goes through the center of the prostate. Almost all prostate cancers are adenocarcinomas. These cancers develop from the gland cells (the cells that make the prostate fluid that is added to the semen). Other types of cancer that can start in the prostate include small cell carcinomas, neuroendocrine tumors (other than small cell carcinomas), transitional cell carcinomas and sarcomas [8]. Despite of good clinical success of cisplatin, it lacks tumor tissue selectivity leading to some severe side effects. Advances in nanotechnology and growing needs in biomedical applications have driven the development of multifunctional nanoparticles [9]. Nanoparticles have the potential to be ideal carriers for delivering anticancer and other therapeutics to diseased sites with minimal collateral damage to normal tissues [10]. Functional copper nanoparticles (Cu NPs) have evoked keen interest in recent decades owing to their size- and shape-dependent optical, catalytic, and therapeutic properties. Copper-based nanomaterial has been notable for excellent therapeutic applications. Functional CuNPs have shown apoptosis-inducing properties through target specific pathways [11]. Copper complexes are used as very effective anticancer agents. This property is associated with the inhibition of DNA replication and mitosis by the addition of Cu complex NPs to DNA strand [12]. This study aimed to evaluate the antitumor activity of Cu complexes NPs against prostate cancer cell lines using advanced biochemical methods.

EXPERIMENTAL

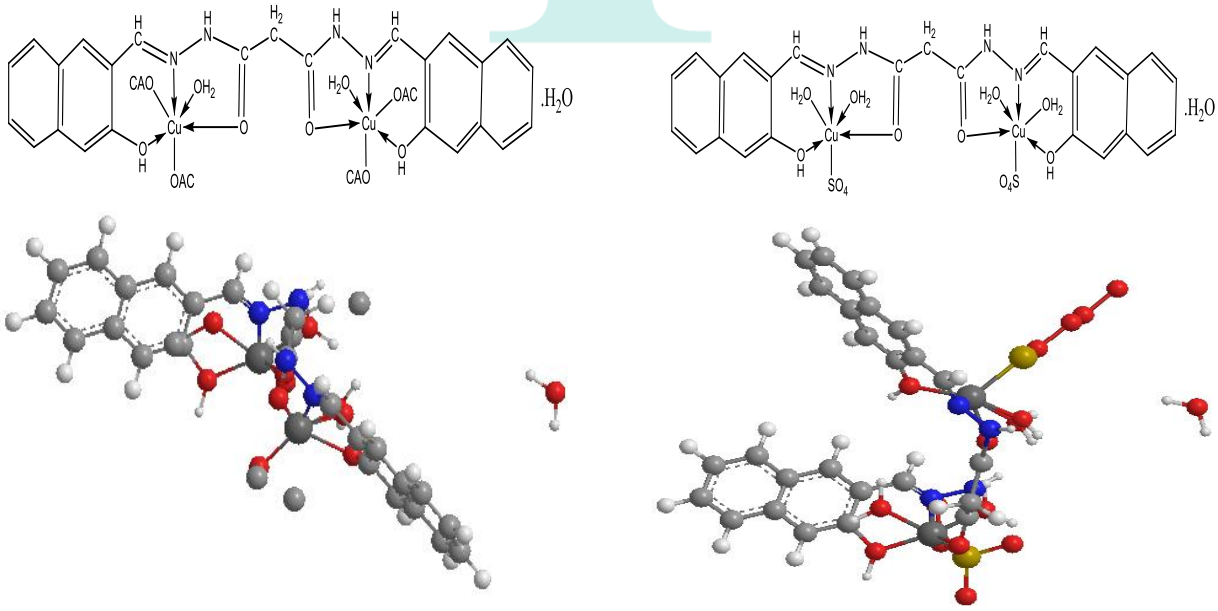
Chemicals

Five different metal complexes of bioactive ligand had been prepared, spectroscopically characterized and purified according to Abdou S. El-Tabl et al, in a previously published article [13]. All solvents and reagents were of analytical grade and used without further purification. The analytical and physical data of the metal complexes are as follows:

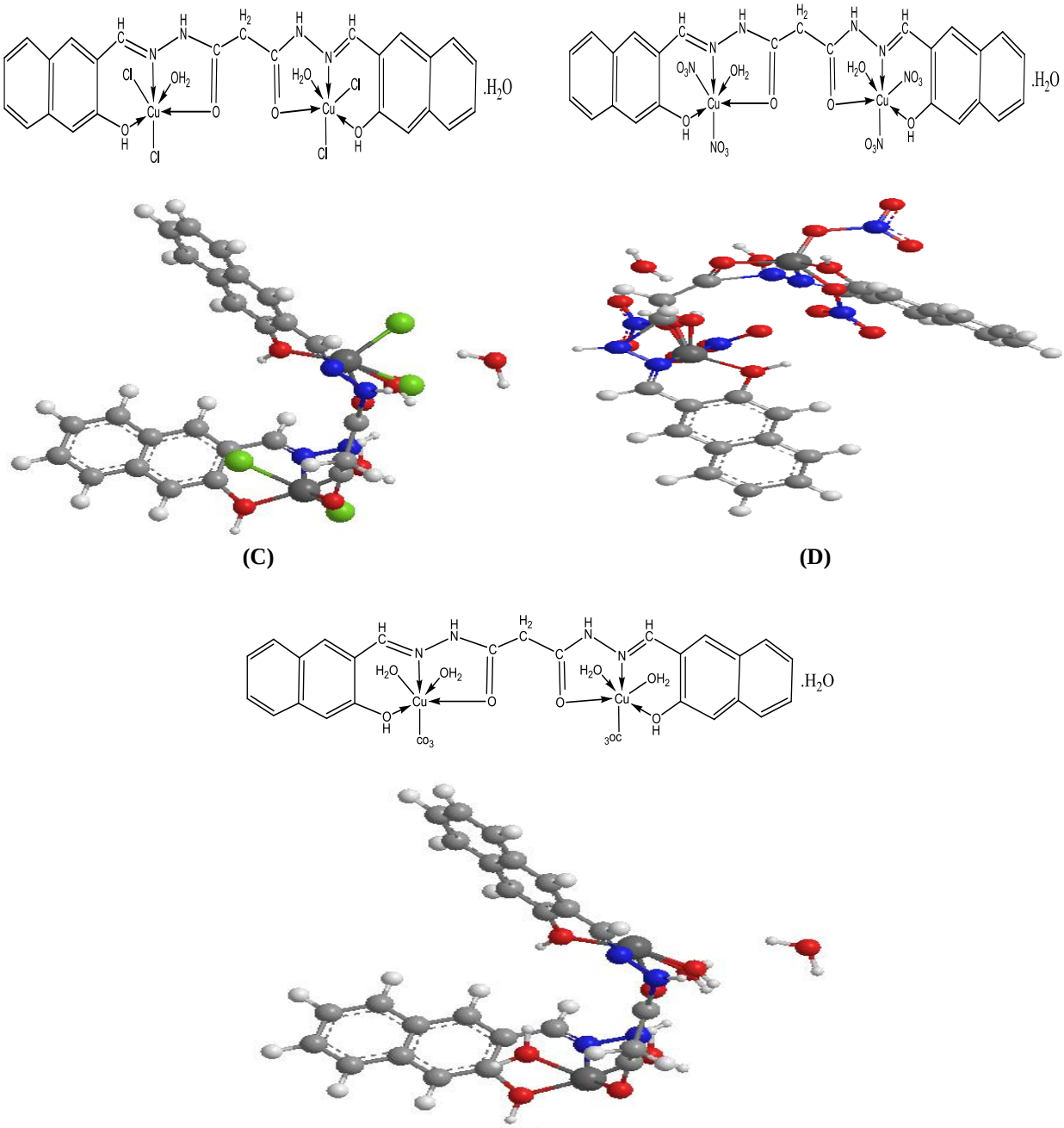
Table 1 Analytical and physical data of the metal complexes

Complexes	Molecular Formula	Color	Molecular weight	Melting point (^{O}C)	Yield (%)
Complex (1)	$C_{33}H_{38}Cu_2N_4O_{15}$	Brown	839.75	> 300	66
Complex (2)	$C_{25}H_{30}Cu_2N_4O_{17}S_2$	Pale yellow	831.73	> 300	80
Complex (3)	$C_{25}H_{26}Cl_4Cu_2N_4O_7$	Green	745.39	> 300	70
Complex (4)	$C_{25}H_{26}Cu_2N_8O_{19}$	Brown	851.59	> 300	67
Complex (5)	$C_{27}H_{30}Cu_2N_4O_{15}$	Greenish brown	843.61	> 300	77

The chemical and 3D structures of the tested complexes were as follow:



(A) **(B)**



(C) **(D)**

(E)

Fig. 1 The chemical and 3D structures of: **(A)** complex **(1)**, **(B)** complex **(2),** **(C)** complex **(3), (D)** complex **(4)** and **(E)** complex **(5)**

Instrumentation and Measurements

Transmission Electron Microscope Characterization (TEM)

TEM samples for colloidal suspension of the complexes in distilled water were prepared by dropping the colloids onto carbon-coated TEM grids and allowed the liquid carrier to evaporate in air then assayed by a JEOL 1400 plus transmission electron microscope [14]**,** Faculty of Science, Alexandria University, Egypt.

***Invitro* studies**

Mammalian cell lines

PC-3 cell line (prostate carcinoma) was obtained from VACSERA Tissue Culture Unit. Giza-Egypt.

Chemicals Used

Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%)

It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with distilled H_2O and filtered through a Whatmann No.1 filter pape

Cell line Propagation

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, for 24 h, the viable cells yield was determined by a colorimetric method [15]. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph-pad Prism software (San Diego, CA. USA) [16].The cellular morphology was observed using an inverted microscope (CKX41; Olympus, Japan) equipped with the digital microscopy camera to capture the images representing the morphological changes compared to control cells. The cytopathic effects (morphological alterations) were microscopically observed at 100x.

Molecular docking studies

Computational simulations enhanced noticeably making it feasible to utilize computational approaches in drug design. The Molecular Operating Environmental module MOE2015 software package is used to predict the biological features of candidate drugs and to anticipate the experimental results [17].

BIOLOGICAL STUDIES

Animals

90 healthy male albino rats of Westar strain, 8 weeks old (180±5 g) were purchased from National Cancer Institute, Cairo, Egypt. Rats were housed in cages at regulated temperature (22- 25 °C). They were kept under good ventilation under a photoperiod of 12-h light/12-h darkness schedule with lights-on from 06:00 to 18:00. They all received a standard laboratory diet (60% ground corn meal, 10% bran, 15% ground beans, 10% corn oil, 3% casein, 1% mineral mixture and 1% vitamins mixture), purchased from Meladco Feed Company (Obor City, Cairo, Egypt) and supplied with drinking water throughout the experimental period.

Acute toxicity study

Determination of the lethal dose 50 (LD_{50}) using experimental animals was done for the studied compounds as described by Akhila et al. [18]. The acute toxicity of the chosen complexes was done on 30 animals (6 animals per group/5 groups). The tested complexes were dissolved in DMSO diluted by sterile saline 0.9% NaCl in a maximum concentration of 0.2% by volume to be able to inject intra-peritoneal. The chosen complexes were administrated with graded doses of 1×10^{-6}, 5×10^{-6}, 1×10^{-5} reached to 1×10^{-4} mmole/L/Kg body weight under the same environmental conditions. Higher concentrations of the tested complexes were not done due to their incomplete dissolution. After administration of the chosen concentrations, the rats were observed for toxic effects after 24h of treatment. The toxicological effects were observed in terms of mortality and expressed as lethal dose 50 (LD_{50}). The LD_{50} for the five complexes nanoparticles were devoid of any toxicity in rats when given the selected different doses by intraperitoneal route.

Experimental design

Animals were allowed 10 days for adaptation. They were then randomly distributed into 6 equal groups, 10 rats each. The animal groups were recognized as follows:

1. Group 1 (Control): Normal healthy animals injected intra peritoneal with 0.2% solution of DMSO dissolved in sterile 0.9% NaCl saline in a maximum concentration of 0.2% by volume for 6 weeks.

2. Group 2, Complex **(1)**: Each animal was injected intra peritoneal with $1x10^{-5}$ mmol/L of the tested complex for 6 weeks.
3. Group 3, Complex **(2)**: Each animal was injected intra peritoneal with $1x10^{-5}$ mmol/L of the tested complex for 6 weeks.
4. Group 4, Complex **(3)**: Each animal was injected intra peritoneal with $1x10^{-5}$ mmol/L of the tested complex for 6 weeks.
5. Group 5, Complex **(4)**: Each animal was injected intra peritoneal with $1x10^{-5}$ mmol/L of the tested complex for 6 weeks.
6. Group 6, Complex **(5)**: Each animal was injected intra peritoneal with $1x10^{-5}$ mmol/L of the tested complex for 6 weeks.

Blood collection

At the end of the experimental period, animals were fasted overnight prior to dissection under light isoflurane anesthesia. Blood was drawn from the venacava and centrifuged at 3000g for 10 min; whole EDTA blood was collected for hematological studies.

Biochemical analyses

Liver enzymes activities, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated using kinetic kits purchased by Human Diagnostics Kits, Germany [19]. The liver functions, albumin concentration, bilirubin (Total and direct) and kidney functions, blood urea and serum creatinine were measured using Diamond Diagnostics kits, Egypt [20]. All biochemical analyses were determined using a Biosystems BTS-310 Spectrophotometer.

Hematological analyses

Hemoglobin (Hb) was determined using Drabkin's solution kit purchased by Vitro Scient, Egypt [21]. Red blood corpuscles count (RBCs), total leucocytes count (TLC) and platelets count (PLTs) were determined manually [22].

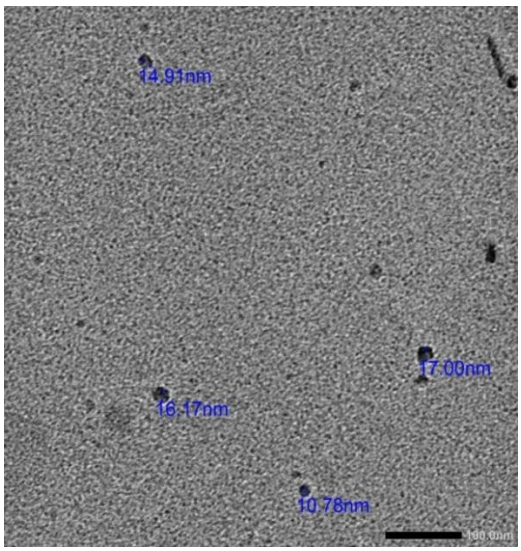
Statistical analysis

Data were subjected to statistical significance tests using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. The statistical analysis was carried out using SPSS 20.00 software. The results were expressed as mean ± SD and the differences were considered significant at $P \leq 0.05$ [23].

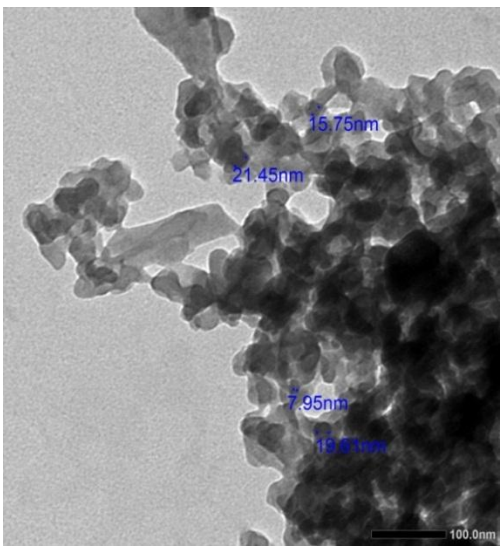
RESULTS

Transmission Electron Microscopy Characterization (TEM)

The average diameter of the tested complexes particles were determined to be 14.715±2.76 nm, 16.19± 5.984, 49.534± 20.919, 4.692± 0.672 and 33.45± 11.637 respectively as shown in Fig. 2. The complexes particles presented in Nano size i.e., presents in a diameter between 1 and 100 nm in size that exhibit new or enhanced size-dependent properties compared with larger particles of the same material with many advantages such as: Increased bioavailability, dose. proportionality, decreased toxicity, smaller dosage form (i.e., smaller tablet), stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-Nano particulate dosage forms, increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body, faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity and reduction in fed/fasted variability [24].



(A)



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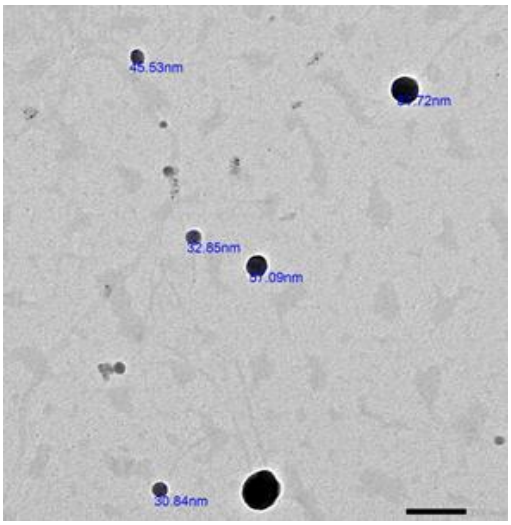

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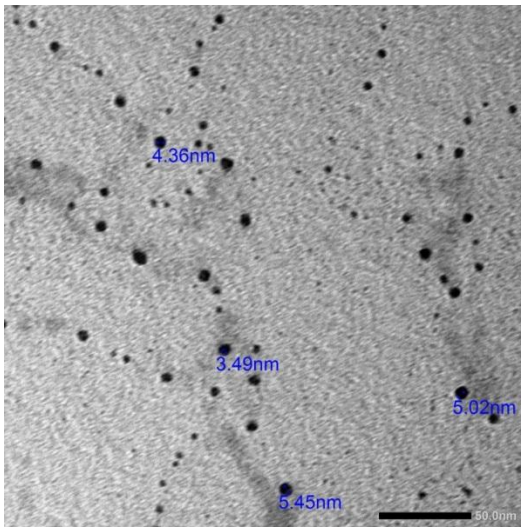

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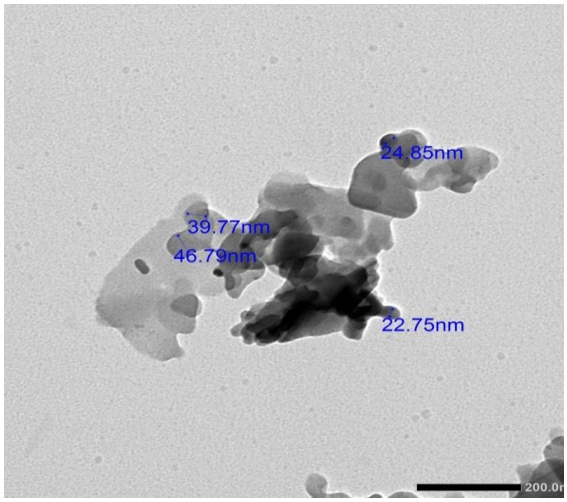
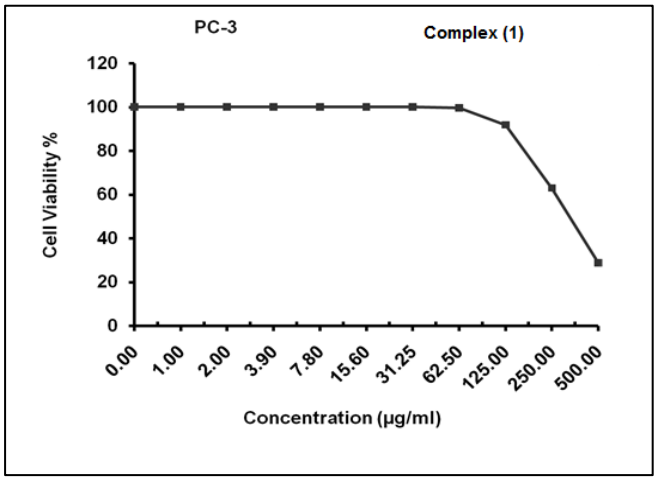


(E)

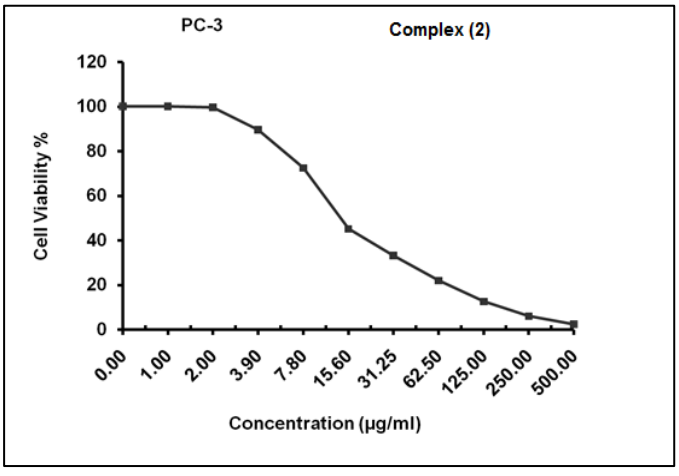
Fig. 2 TEM images for: **(A)** complex **(1)**, **(B)** complex **(2),**
(C) complex **(3), (D)** complex **(4)** and **(E)** complex **(5)** nanoparticle

Evaluation of cytotoxicity against PC-3 cell line

Cytotoxicity results indicated that the tested complexes NPs had IC_{50} of 344.46 ± 14.65 µg/ml for complex **(1)**, 14.19 ± 0.93 µg/ml for complex **(2),** 7.76 ± 0.46 µg/ml for complex **(3)**, 59.93 ± 3.98 µg/ml for complex **(4)** and 11.40 ± 0.57 µg/ml for complex **(5)**. The results demonstrated a potent cytotoxicity of complexes (**2, 3** and **5**) against PC-3 prostate cancer cell line but weak one in case of complexes (**1** and **4**), whereas the standard drug cisplatin had IC50 = 5.09± 0.31 µg/ml.



(A)



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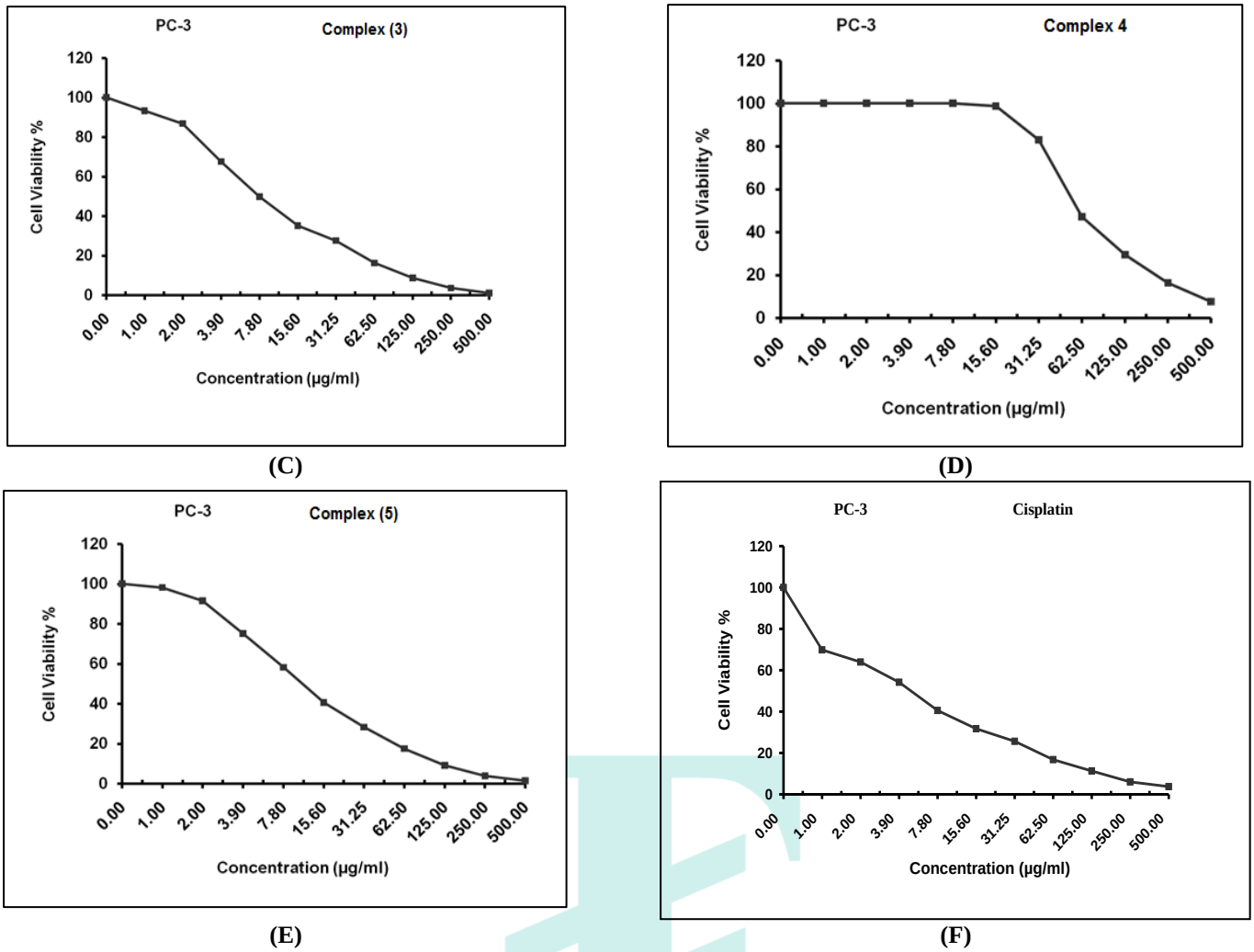


Fig. 3 Cytotoxicity of **: (A)** complex **(1)**, **(B)** complex **(2), (C)** complex **(3), (D)** complex **(4)**, **(E)** complex **(5)** nanoparticle and **(F)** standard drug, cisplatin against PC-3 cell line

The obtained data indicated the surviving fraction ratio against PC-3 tumor cell line increasing with the decrease of the concentration in the range of the tested concentrations. This can be explained as some metal ions bind to DNA. It seems that, changing the anion and the nature of the metal ion has effect on the biological behavior, due to alter binding ability of DNA binding, so testing of different complexes is very interesting from this point of view. Also, the positive charge of the metal increases the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhance the biological activity [25]. Moreover, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction [26].

Molecular docking studies

The simulated interaction of designed drug with the protein structure of selected pathogens was modeled by MOE 2015.10 program. The 3D crystal structure of the selected proteins was obtained from the Protein Data Bank (PDB). The inhibition efficiency of the designed drugs is evaluated by the strength of interactions with the target proteins, which was predicted from the scoring energy and the length of the H-bonds in the docked complex [27, 28], removal of water molecules, atomic charges clarifying, and then energy minimization by MMFF94x force field .10 Poses of interactions were recorded for each species, where the best pose with the shortest ligand-receptor distance and the highest scoring energy is presented in the results and discussion section.

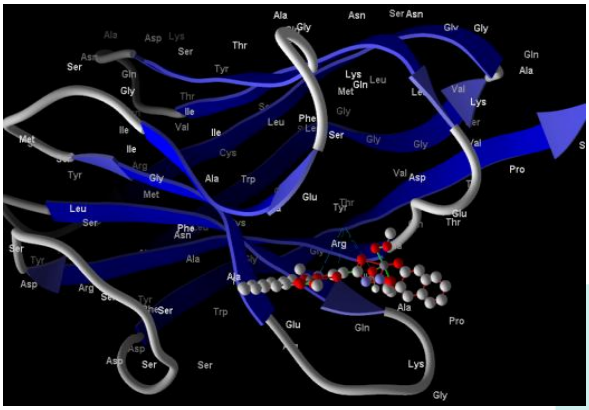
In the present study, the docked copper metal complex **(1)** with 6XXO which is responsible for prostate cancer showed an excellent electrostatic and hydrogen bond between ligand and receptor interaction distances were ≤ 3.5 A in most cases, which indicates the presence of typical real bonds which means high binding affinity . For example, the nearest interaction is observed via H-donors with 6XX0 (2.30A) and metal complex **(1)** with scoring energy (S) -0.5981 kcal , nine binding sites of designed drug with different amino acids (Arg33,Glu 46, Ala 47,Glu 89 and Lys 43) were observed which demonstrating their higher inhibition.

The designed drug copper metal complex **(2)** was docked with 6XXO prostate cancer prostate showed an excellent electrostatic and hydrogen bond between ligand and receptor. The nearest interaction is observed via H-donors with 6XX0 (2.30A) and complex **(2)** which indicates the presence of typical real bonds which means high binding affinity with scoring energy (S) -0.5981 kcal, seven binding sites of designed drug with different amino acids (Arg 38, Ala 40, Ala 47, Ala 61, Glu 46 and Val 48) were observed which demonstrating their higher inhibition.

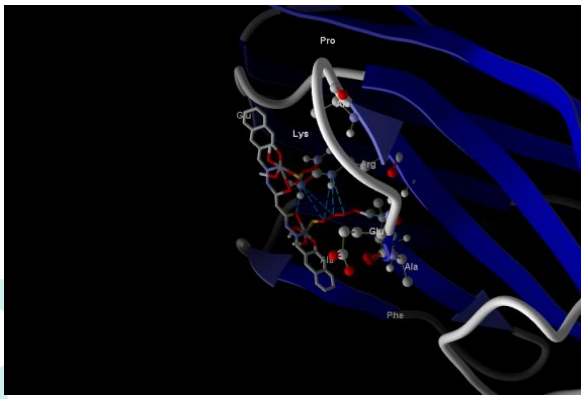
Complex **(3)** was docked with 6XXO prostate cancer and showed a weak electrostatic and hydrogen bond between ligand and receptor through one binding site .The nearest interaction is observed *via* H-donors with 6XX0 (2.50A) and metal complex (3) which indicated the presence of typical real bonds With scoring energy (S) -2.5 kcal, only one binding sites of designed drug with different amino acids (Arg 38) were observed which demonstrating lower inhibition for 6XXO.

However the designed drug copper metal complex (4) was docked with 6XXO prostate cancer prostate showed an excellent electrostatic and hydrogen bond between ligand and receptor. The nearest interaction is observed via H-donors with 6XX0 (2.33A) and (copper metal complex23) which indicates the presence of typical real bonds which means high binding affinity With scoring energy (S) -0.2626 kcal , Nine binding sites of designed drug with different amino acids(Arg 38, Ala 40, Ala 47 and Glu 46) were observed which demonstrating their higher inhibition.

While the docked metal complex **(5)** with 6XXO prostate protein cancer showed a very good electrostatic and hydrogen bond between ligand and receptor interaction distances were $\leq$ 3.5 A in most cases, which indicates the presence of typical real bonds which means high binding affinity. For example, the nearest interaction is observed via H-donors with 6XX0 (3.08A) and copper metal complex **(5)** with scoring energy (S) -1.7878 kcal , nine binding sites of designed drug with different amino acids (Arg33,Glu 46, Ala 47 and Phe 80) were observed which demonstrating their higher inhibition.



(A)



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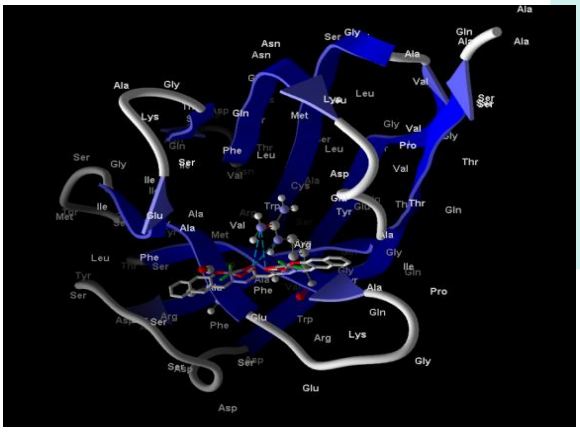

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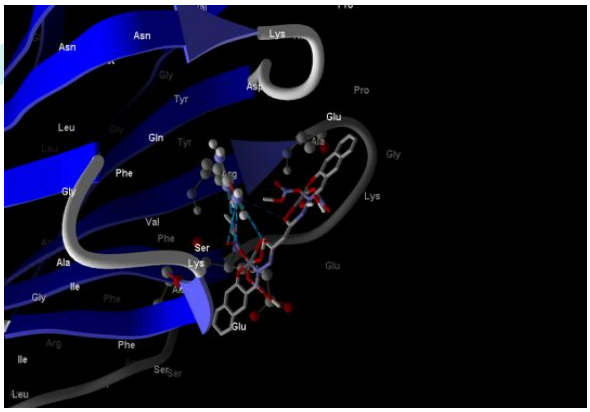

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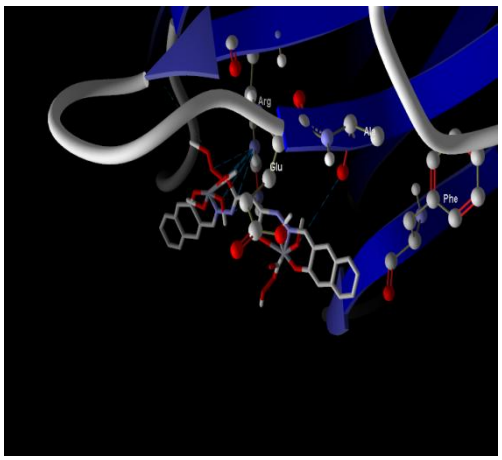

(E)

Fig. 4 Virtual molecular docking of the docked AR protein with: **(A)** complex **(1)**, **(B)** complex **(2),** **(C)** complex **(3), (D)** complex **(4)** and **(E)** complex **(5)**

Biological studies "*Invivo* studies"

Biochemical analyses

The results of the present study [Table 1] recorded that the measurements of liver functions (AST, ALT, ALP, albumin and total bilirubin), renal functions (B. Urea & S. Creatinine) showed no significant differences between treated groups by the chosen complexes and the control group, which proves that there are no toxic side effects for the tested complexes.

Table 2 Statistical analysis (ANOVA) for liver and kidney function tests in the different groups

Parameters	Control	Complex (1)	Complex (2)	Complex (3)	Complex (4)	Complex (5)
AST (U/l)	97.55±16.221	101.07±18.34	96.963±15.117	101.07±10.115	105.23±9.649	104.63±12.2
ALT (U/l)	32.964±6.118	31.227±5.920	32.441±8.221	30.70±4.089	34.565±4.955	35.05±4.71
ALP (U/l)	137.284±20.957	140.01±12.27	137.229±17.005	132.69±16.217	139.2±18.24	139.93±14.9
Alb (g/dl)	4.56 ±0.927	4.501 ±0.532	4.492 ±0.337	4.720±0.217	4.325±0.217	4.448±0.145
T. Bilirubin (mg/dl)	0.477±0.117	0.451±0.082	0.445±0.201	0.481±0.094	0.474±0.038	0.449±0.037
B. Urea (mg/dl)	31.227±2.898	30.714±4.353	29.997±2.551	33.004±5.01	31.633±1.977	32.716±3.93
S. Creatinine (mg/dl)	0.521 ±0.124	0.517 ±0.144	0.501 ±0.069	0.538±0.0934	0.526±0.107	0.493±0.062

ANOVA: analysis of variance; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alb: albumin; ALP: alkaline phosphatase; T. Biliribin: Total bilirubin; Each value is represented as mean ± SD. SD: standard deviation. Each value is represented as mean ± SD. Data with different superscripts are significantly different at $p \leq 0.05$.

Hematological Analysis

The results of the analysis of some hematological parameters (Hb, RBCs, TLC and platelets counts) showed no significant changes between the control group and the treated groups by the chosen complexes as follow:

Table 3 Statistical analysis (ANOVA) for hematological tests in the different groups treated by Cu complex NPs

Parameters	Control	Complex(1)	Complex (2)	Complex (3)	Complex (4)	Complex (5)
Hb (g/dl)	16.40 ±0.378	16.00±0.704	15.866±1.132	16.05±1.769	15.733±0.70	15.883±0.592
RBCs (X 10^6/cmm)	6.551 ±0.449	6.248±0.398	6.087±0.628	6.327±1.155	6.01±0.571	5.991±1.051
TLC (X 10^3/cmm)	9.978±0.969	9.72±1.183	8.589±0.637	9.12±0.927	8.892±0.789	9.267±0.664
PLTs (X 10^3/cmm)	559.15±55.21	575.50±92.03	539.158±36.99	602.21±44.58	598.25±26.8	575.7±28.361

ANOVA: Analysis of variance; Hb: Hemoglobin concentration; RBCs: Red blood corpuscles count; T.L.C: Total leucocytic cont; PLT: Platelets count. Each value is represented as mean ± SD. SD: standard deviation Data with different superscripts are significantly different at $p \leq 0.05$.

DISCUSSION

This study was conducted to evaluate the efficiency, the biological activity and the cytotoxic effects of five synthesized Cu (II) complexes NPs against human prostate cancer cell line (PC-3 cells), *invitro* study. Results showed potent effect of Cu complex NPs in comparison with the standard drug (Cisplatin) in a dose-dependent manner, where increasing concentration of Cu complex NPs resulted in increased percentage of dead cells. This result proving that the tested complex NPs inhibit cell proliferation via induction of apoptotic cell death. In addition, Cu complex NPs produce a cytotoxic effect by reducing cell viability and causing inter-nucleosomal DNA fragmentation, G2/M cell-cycle arrest, and hypo-diploid accumulation emphasizing that the Cu complex NPs have potential anticancer properties and can be applied as cancer therapeutics. Docking results include ligand-receptor sites, interaction type, interaction distances (A), internal energy (E), and scoring energy (S) in kcal/mole. The negative value for energies implies the spontaneous binding of the tested inhibitor to the target protein. A previous study showed that Cu complex NPs' uptake by the cells endocytosis and emphasized intracellular release of Cu^{+2} ions from Cu complex NPs (that) blocks cell division by binding to DNA causing DNA damage and contributed to the cytotoxicity and metabolic stress activating cell death via apoptosis. Also, down regulation of proliferating cell nuclear antigen, a factor critical for DNA replication and repair following Cu NP treatment, supports the anti-proliferative effects of Cu complex NPs [29]. The present biochemical and hematological results revealed that the measurements of liver functions, renal functions and different hematological parameters showed no significant differences between treated groups by the chosen complexes and the control group, which proves that there are no toxic side effects for the tested complexes.

CONCLUSION

Reports indicate that Cu complex NPs might be useful as therapeutics in cancer therapy, and Cu complex NPs in combination with Hadron therapy led to an enhancement of strongly lethal DNA damage caused by double-strand breaks. A previous study showed that Cu complex NPs uptake by the cells endocytosis and emphasized intracellular release of Cu^{2+} blocks cell division by binding to DNA causing DNA damage and contributed to the cytotoxicity and metabolic stress activating cell death via apoptosis [30]. Cu complex NPs produces a cytotoxic effect by reducing cell viability and causing inter-nucleosomal DNA fragmentation, G2/M cell-cycle arrest, and hypo-diploid accumulation emphasizing that they have potential anticancer properties and can be applied as cancer therapeutics [31]. Also, down regulation of proliferating cell nuclear antigen, a factor critical for DNA replication and repair following Cu NPs treatment, supports the anti-proliferative effects of Cu complex NPs [32]. The tested complexes NPs were thought to serve as a reservoir for metal ions that can induce DNA damage in cancer cells [33].

AUTHOR'S CONTRIBUTIONS

ASE designed the study and performed the complexes synthesis. AMA, MMA and AGA performed most of the experiments (Biochemical and hematological analyses), analyzed and interpreted the data. SAK performed and interpreted the molecular docking studies. AMA and AGA wrote the first version of the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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ETHICS APPROVAL

This study was performed after getting permission from the Institutional Animal Ethical Committee, Menoufia University, Egypt (Approval ID: MUFS/S/BIO/7/23).

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