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The Cytotoxicity of Some Biologically Active Nano Compounds against Colon Cancer:
Advanced Biochemical Analyses

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Abstract
Colorectal cancer is one of the most common cancers worldwide, and it is also one of the major causes of mortality from cancer. Chemotherapy drugs are generally limited due to various complications, as well as the development of resistance and recurrence. This study determined the selective cytotoxicity and mutagenic potential of five novel metallo-organic complexes nanoparticles against colon cancer cell line (HCT-116). The complexes have been prepared and then their structures were characterized by some instrumental analyses. The electron microscope data confirmed the Nano form of these complexes. *In vitro* antitumor activity of the complexes had been studied against colon cancer cell lines and the IC_{50} values were detected. The cytotoxicity studies of the complexes showed that, the complexes have no side effects after completing the experiment period (6 weeks). The more effective and probable binding modes between the studied metal complexes NPs with different active sites of Colorectal cancer were investigated using molecular docking studies. The results augur well for colorectal cancer treatment.

Keywords
Colorectal cancer, Cytotoxicity, Mutagenic potential, HCT-116 cell lines and molecular docking

INTRODUCTION
Colorectal cancer (CRC) is the third highest prevalence rate of all cancer types worldwide, with an expected 2.4 lakhs incidences by 2035 [1, 2]. Surgery, chemotherapy or radiotherapy, immunotherapy, hormonal therapy, and pharmacotherapy are all used to treat CRC, focusing on the tumor site and stage of the disease [3]. Apoptosis prevents damaged cells from developing out of balance under normal physiological conditions. However, secondary mutations in apoptosis-regulating genes may allow these cells to evade the regulatory mechanisms of apoptosis. Cancer chemoprevention is described as the use of chemical or organic compounds substances to prevent, suppress, or counteract tumor genesis or the growth of metastatic carcinoma [4]. Almost all modern therapeutic medicines have their sources in herbal medicine, because they're either unaltered dietary supplements or their better synthetic analogues [5]. Bioactive compounds have been modified to improve therapeutic effectiveness, bioavailability, specificity, as well as a variety of many features, including the implementation of some potential chemotherapeutic agents [6, 7, 8]. The rational design of

effective anticancer agents now requires the development of molecular targets associated with cancer metabolism [9, 10], [11]. Several researches have examined into the various functions played by the several biological signaling molecules (factors) involved in cancerous cell growth and expansion [12, 13]. Therapeutic potential of metal complexes in cancer therapy has attracted a lot of interest mainly because metals exhibit unique characteristics, such as redox activity, variable coordination modes and reactivity toward the organic substrate [14]. These properties become an attractive probe in the design of metal complexes that selectively bind to the biomolecular target with a resultant alteration in the cellular mechanism of proliferation [15]. Several metal-based compounds have been synthesized with promising anticancer properties, some of which are already in use in clinical practice for diagnosis and treatment while some are undergoing clinical trials [16]. Metal-based compounds synthesized recently are products of drug design targeted at achieving specific objectives that the original compound could not achieve and such compounds exhibit a different spectrum of cytotoxicity [17]. Metal complexes are used as very effective anticancer agents. This property is associated with the inhibition of DNA replication and mitosis by the addition of metal complex NPs to DNA strand [18]. This study aimed to evaluate the antitumor activity of some complexes NPs against colorectal cancer cell lines using advanced biochemical methods.

EXPERIMENTAL

Chemicals and Instrumentations

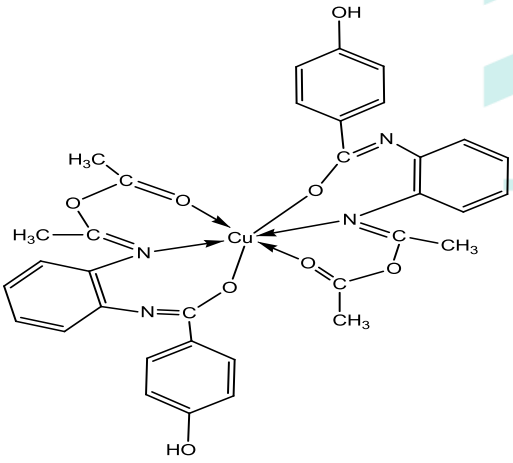
All solvents and reagents were of analytical grade and used without further purification.

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 [19]. All solvents and reagents were of analytical grade. The analytical and physical data of the metal complexes are as follows:

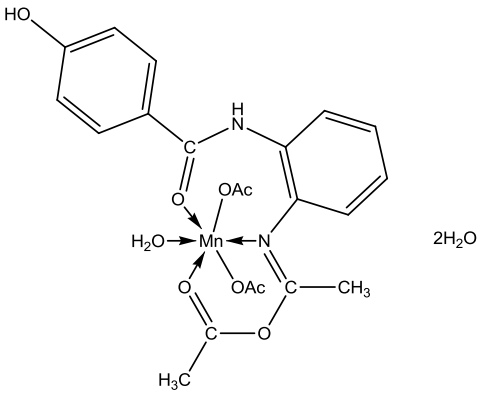
Table 1 Analytical and physical data of the metal complexes

Complexes	Molecular Formula	Color	Molecular weight	Melting point (^{O}C)	Yield (%)
Complex (1)	$C_{34}H_{30}CuN_4O_8$	Black	685.5	93	95
Complex (2)	$C_{21}H_{28}MnN_2O_{11}$	Brown	538.93	87	60
Complex (3)	$C_{17}H_{24}MgN_2O_{12}S$	Light Brown	504.31	75	70
Complex (4)	$C_{17}H_{20}PbN_4O_{12}$	Orange	679.2	93	87
Complex (5)	$C_{17}H_{20}ZnN_4O_{12}$	Orange	537.39	80	68

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



(A)



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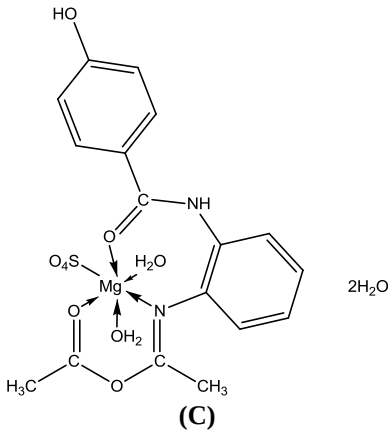
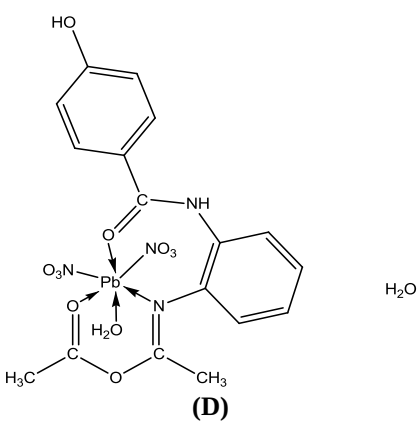
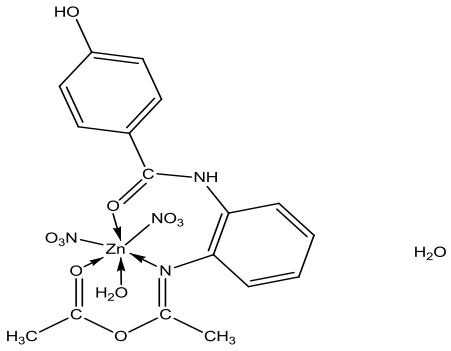


(C)



(D)



(E)

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

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 [20]**,** Faculty of Science, Alexandria University, Egypt.

***In vitro* studies**

HCT-116 cells (human colon cancer cell line) were obtained from VACSERA Tissue Culture Unit), Giza-Egypt. 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%), 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 paper. 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 [21]. 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 [22]. 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) [23].

Molecular docking studies

The Molecular Operating Environmental module MOE2015 software package is used to predict the biological features of candidate drugs and to anticipate the experimental results [24]. NPACT (http://crdd.osdd.net/raghava/npact/) is a curated data base of plant originated natural compounds that show antitumor activity. It have 1574 access and every record gives details on their structure properties (physical, elemental and topological) cancer type, cell lines, inhibitory values (IC_{50}, ED_{50}, EC_{50}, GI_{50}), molecular targets, commercial suppliers and drug likeness of compounds [25]. Among the total entries we retrieved only 543 compounds were reported for colorectal cancer. SMILES "Simplified Molecular Input Line Entry System" format of those compounds were taken from NPACT and convert as PDB format using Online Smiles Translator. All the compounds were loaded using input molecule option and were energy minimized with the MM2 process and changed to pdb. Extension file which is readable at the ADT interface.

Biological studies

Animals

90 healthy male albino rats of Wistar strain, 8 weeks old (170 - 200 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 lethal dose 50 (LD_{50}) using experimental animals was done. 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} and reached to 1×10^{-4} mmol/L/Kg body weight under the same environmental conditions. More doses were not possible due to poor solubility in case of higher concentrations. 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}) [26]. The LD_{50} for the five complexes nanoparticles were devoid of any toxicity in rats when given the selected different doses by intra-peritoneal route, therefore, the therapeutic dose was set at one tenth of the highest concentration reached [27].

Experimental design

60 albino rats of Wistar strain were allowed 10 days for adaptation. The animals 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 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 Diagnostic Kits, Germany [28]. The liver functions, albumin concentration, bilirubin (Total and direct) and kidney functions, blood urea and serum creatinine were measured using Diamond Diagnostic kits, Egypt [29]. All biochemical analyses were determined using a Biosystems BTS-350 Spectrophotometer.

Hematological analyses

Determination of hemoglobin (Hb) using Drabkin's solution, red blood corpuscles count (RBCs), total leucocytes count (TLC) and platelets count (PLTs) were determined manually [30].

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 $P \leq 0.05$ [31].

RESULTS

Transmission Electron Microscopy Characterization (TEM)

The average diameter of the tested complexes particles were determined to be 14.715±8.784 nm, 41.928± 10.483, 22.486± 4.008, 18.882± 2.474 and 25.204± 3.608 respectively as shown in **Fig. 2**. The complexes particles presents in a diameter between 1 and 100 nm in size i.e., presented in Nano size that exhibit enhanced size-dependent properties compared with larger particles of the same material with many advantages.

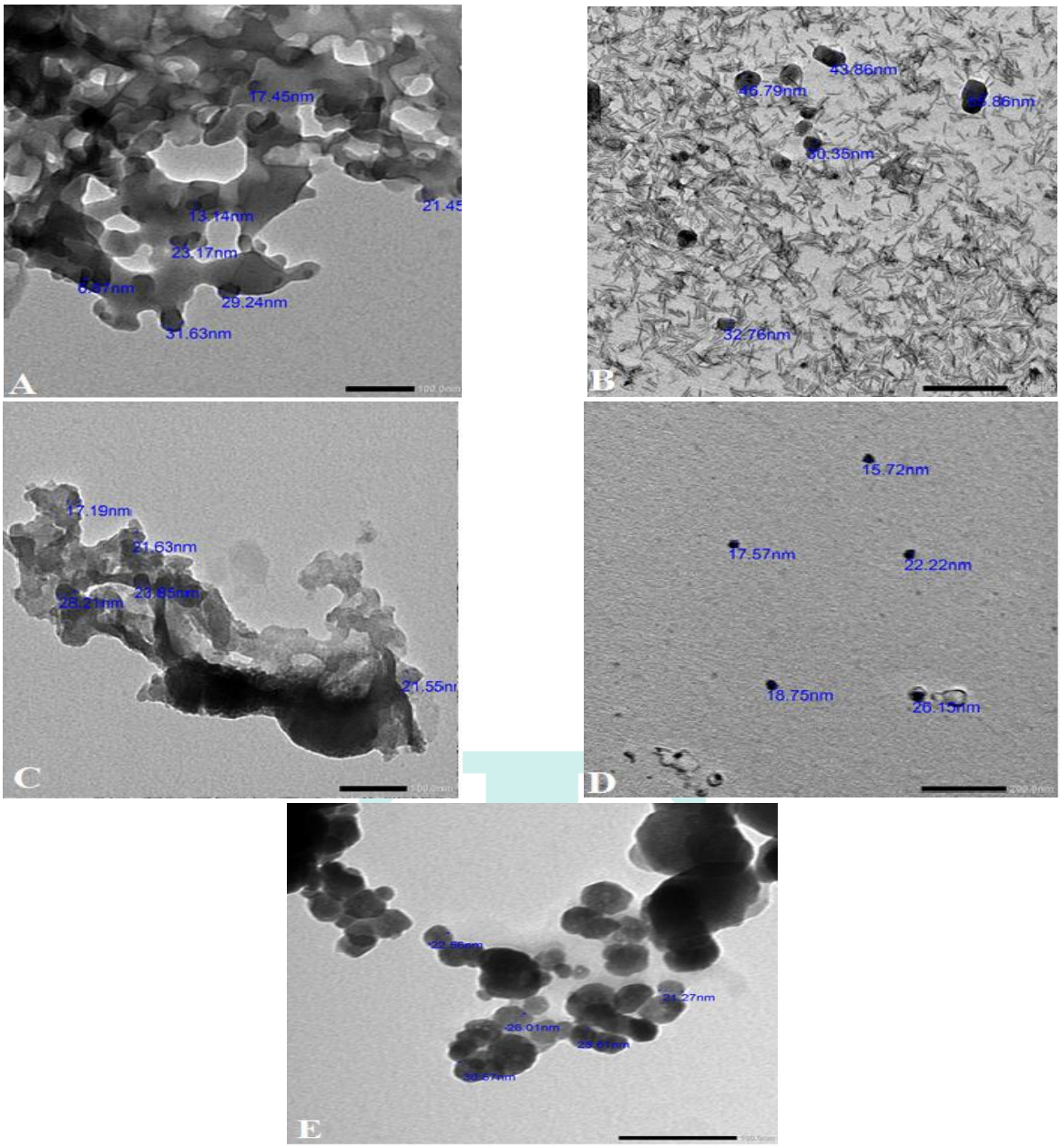
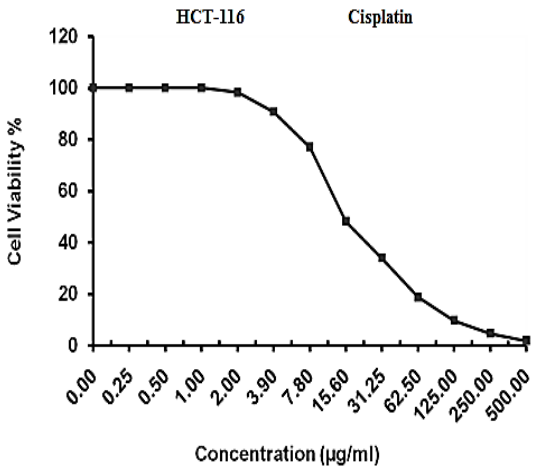


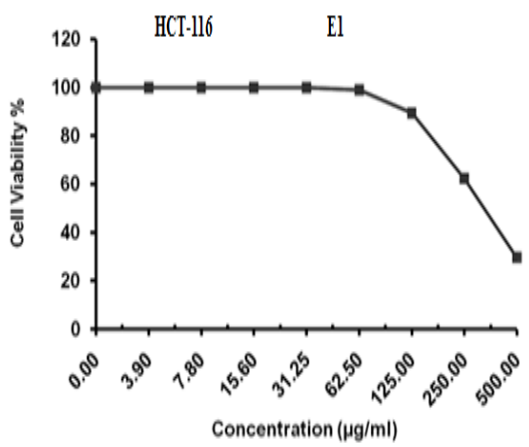
Fig. 2 TEM images for: **(A)** complex **(1)**, **(B)** complex **(2), (C)** complex **(3), (D)** complex **(4)** and **(E)** complex **(5)** nanoparticle

Evaluation of cytotoxicity against A-549 cell line

Cytotoxicity results indicated that the tested complexes NPs had IC_{50} of 344.14 ± 14.03 µg/ml for complex **(1)**, 196.78 ± 7.91 µg/ml for complex **(2),** 18.89 ± 1.89 µg/ml for complex **(3)**, 15.10± 0.75 µg/ml for complex **(4)** and 186.13 ± 6.23 ***µ***g/ml for complex **(5)**. The results demonstrated a potent cytotoxicity of complexes (**3** and **4)** against HCT-116 colon cancer cell line but weak one in case of complexes (**1, 2** and **5**), whereas the standard drug cisplatin had IC_{50} = 14.87± 1.04 µg/ml.



(A)



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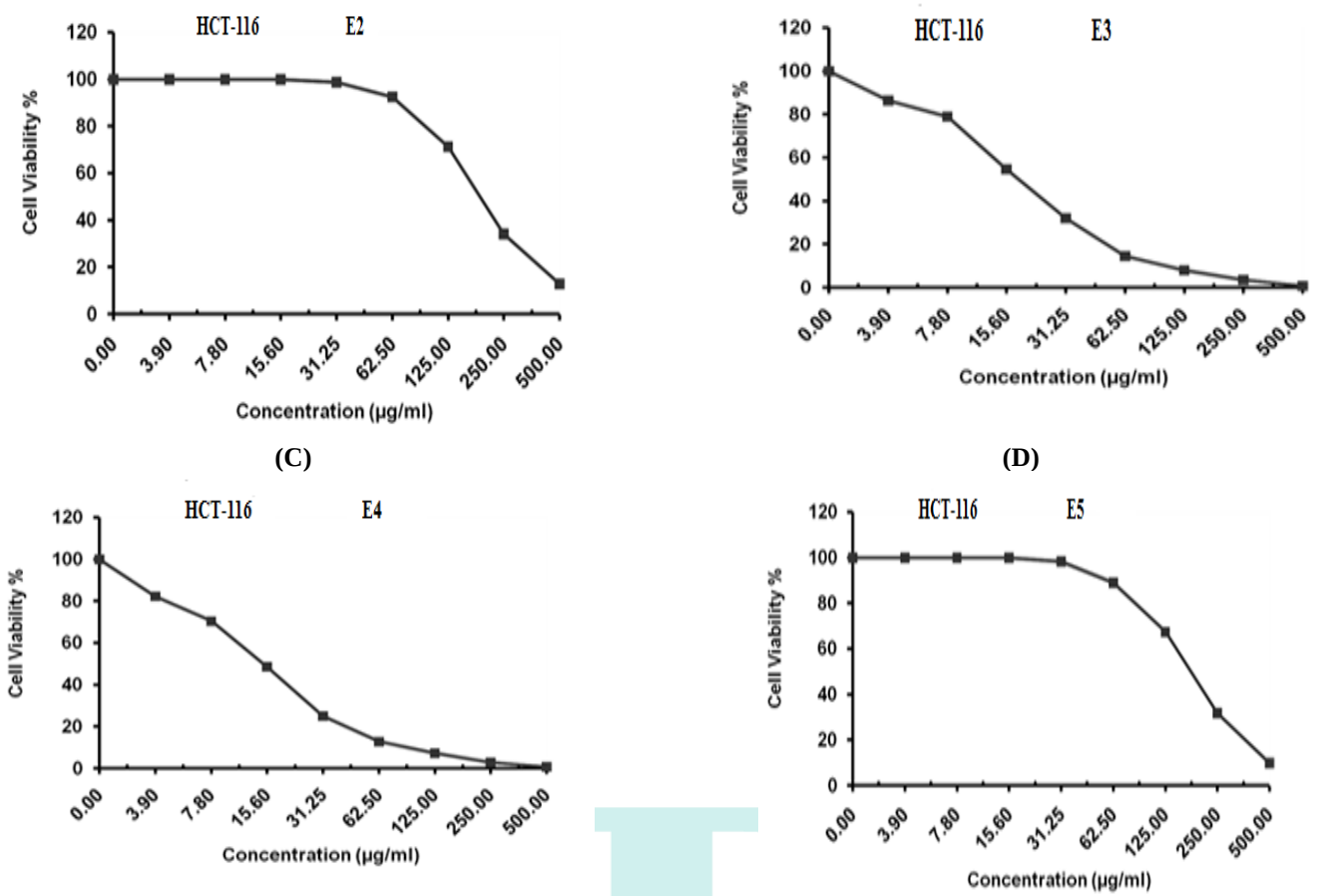
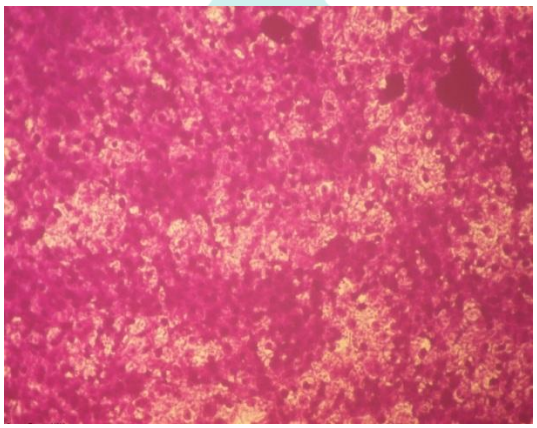
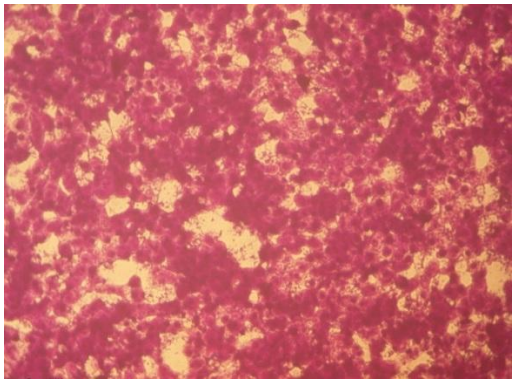


Fig. 3 Cytotoxicity of: **(A)** standard drug, cisplatin, **(B)** complex **(1)**, **(C)** complex **(2), (D)** complex **(3), (E)** complex **(4)**, and **(F)** complex **(5)** nanoparticles against HCT-116 cell line

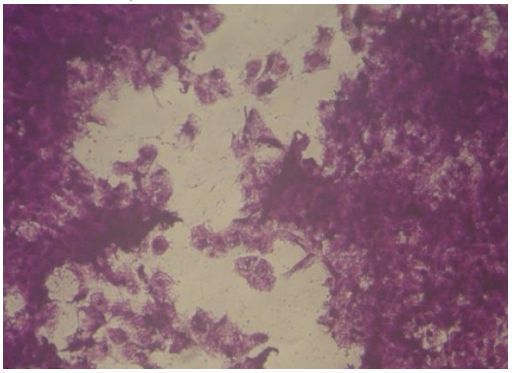
The microscopic histograms showed the chemotherapeutic activity of the tested complex NPs. There is decreasing in the number of available cells. Most of the remaining observed degeneration changes in the form of irregulatory cell membrane opaque and not well formed chromatin regulated of swalling cytoplasm , other showed optatic change in the formed of chrunked cells and increase eosinophilia cells, and picknitoic nucleus as shown in **Fig. 9**



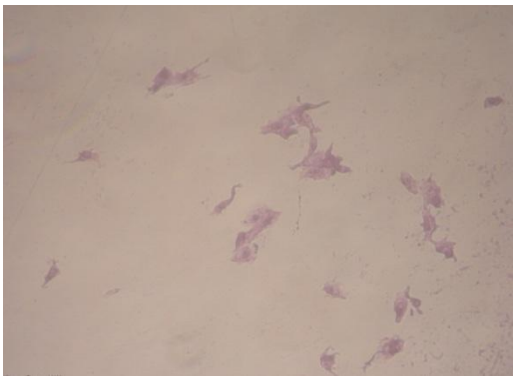
HCT-116 cells (Non-treated control)



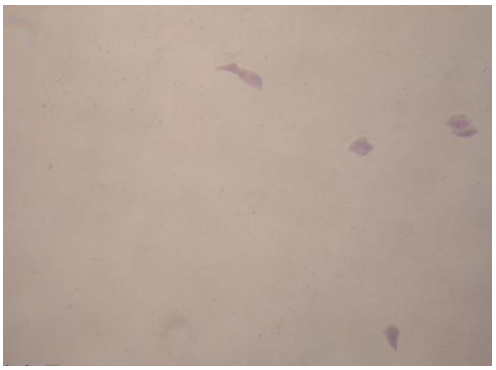
HCT-116 cells treated by complex **(3)** at conc.7.8 µg/ml



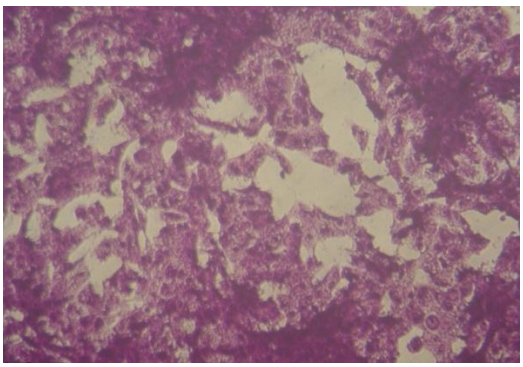
HCT-116 cells treated by complex **(3)** at conc. 31.25 µg/ml



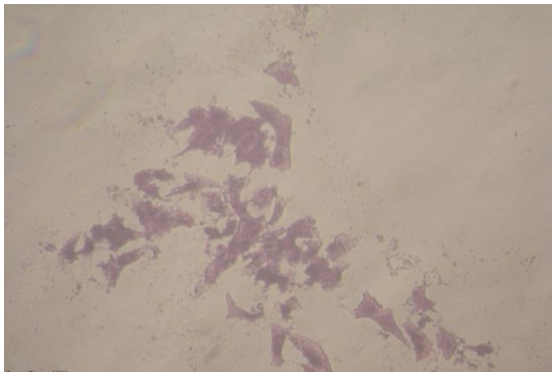
HCT-116 cells treated by complex **(3)** at conc. 125 µg/ml



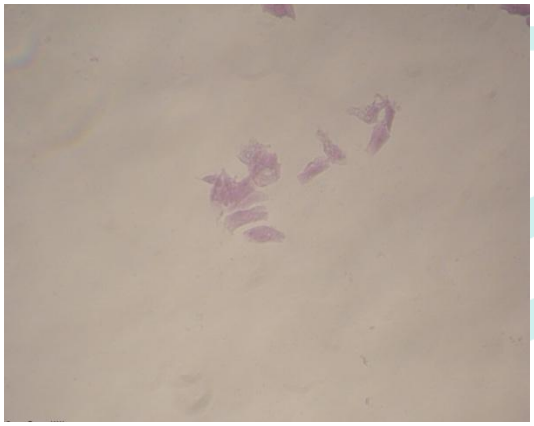
HCT-116 cells treated by complex **(3)** at conc. 500 µg/ml



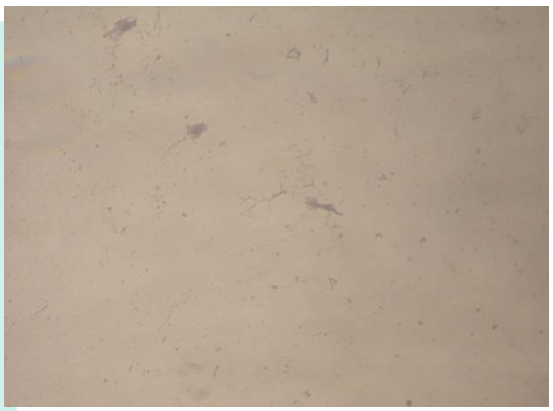
HCT-116 cells treated by complex **(4)** at conc. 7.8 µg/ml



HCT-116 cells treated by complex **(4)** at conc. 31.25 µg/ml



HCT-116 cells treated by complex **(4)** at conc. 125 µg/ml



HCT-116 cells treated by complex **(4)** at conc. 500 µg/ml

Fig. 4 Morphological evaluation of cytotoxicity against HCT-116 colon carcinoma cell line for the highly effective complexes, complex **(3)** and **(4)**

The obtained data indicated the surviving fraction ratio against HCT-116 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. Chemotherapeutic activity of the complex may be attributed to the central metal atom which was explained by Tweedy's chelation theory [33]. 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 [34]. Moreover, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction [35].

Molecular docking studies

In the present study, the protein structures of Traf2 and Nck were used as the receptors docked with the five tested complexes as inhibitors for lung cancer. Before the docking process, the preparation of the pre-protein structure was achieved by removing water molecules and adding polar hydrogen with the MMFF94x force field. Docking results include ligand-receptor sites, interaction type, interaction distances (A), internal energy (E), and scoring energy (S) in kcal/mole [36].

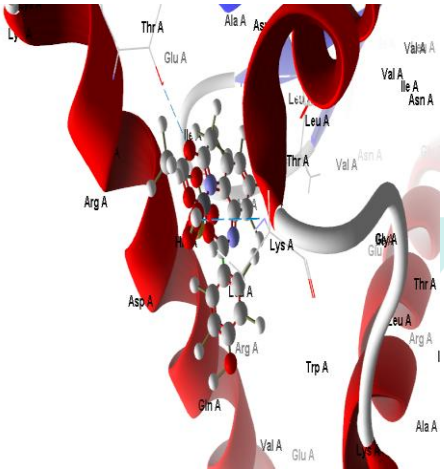
The docked Complex **(1)** "E1" Fig.10, complex have effective ligand-receptor interaction distances were ≤ 3.7 A in most cases, which indicates the presence of typical real bonds and hence high binding affinity. For example, the nearest interaction is observed via H-donors with 2x7x (3.07A) and Complex **(1)** figure With Mol dock score 46475 kcal Furthermore, six binding sites were observed of different amino acids (Lys 121(A),Leu 116(A) and Thr 309(A) with Complex (16) "E1"Figure complex demonstrating their excellent inhibition.

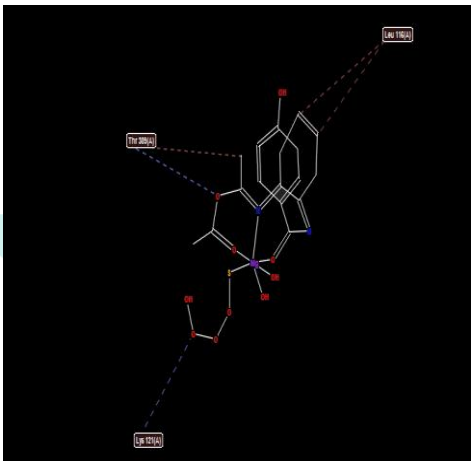
While the docked Complex **(2)** "E2" Fig. 11, complex have effective ligand-receptor interaction distances were ≤ 3.4A in most cases, which indicates the presence of typical real bonds and hence high binding affinity. For example, the nearest interaction is observed via H-donors with 2x7x (1.97A) and " Complex (3) "E2"With Mol dock score 150601 kcal Furthermore, eighteen binding sites were observed of Ser 264 (E) and Ser 268 (E) amino acids

However the docked complex **(3)** "E3" Fig. 12**,** complex have effective ligand-receptor interaction distances were ≤ 3.16A in most cases, which indicates the presence of typical real bonds and hence high binding affinity . For example, the nearest interaction is observed *via* H-donors with 2x7x (2.68A) and "Complex **(3) "E3"**With Mol dock score 73703 kcal Furthermore, four binding sites were observed of different amino acids (Gly 108(A), Glu 183(A) and Thr 182(A))

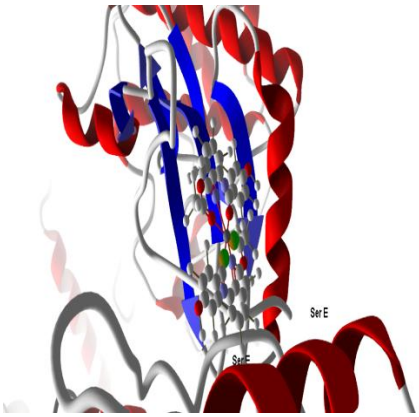
While the docked Complex **(4) "E4"** Fig. 13, complex have effective ligand-receptor interaction distances were ≤ 3.10A in most cases, which indicates the presence of typical real bonds and hence high binding affinity. For example, the nearest interaction is observed *via* H-donors with 2x7x (2.61A) and complex **(4) "E4"**With Mol dock score 73689 kcal Furthermore, four binding sites were observed of different amino acids(Lys 121 (A), Leu 126 (A), Lys 128(A) Thr 124(A) and Thr 120(A))

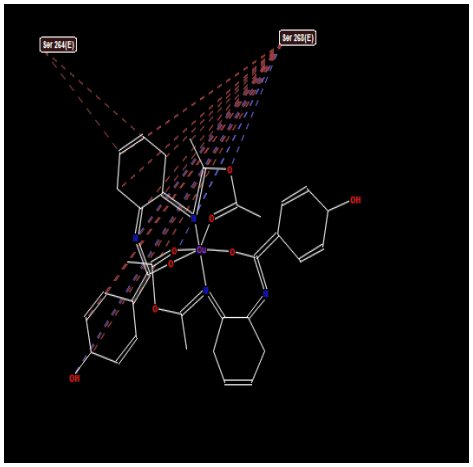
While the docked Complex **(5)** "E5" Fig. 14, complex has effective ligand-receptor interaction distances were ≤ 3.36A in most cases, which indicates the presence of typical real bonds and hence high binding affinity. For example, the nearest interaction is observed via H-donors with 2x7x (2.71A) and Complex (18) "E12" With Mol dock score 78624 kcal Furthermore, four binding sites were observed of different amino acids (His 305 (A), Leu 116 and Thr 305(A).



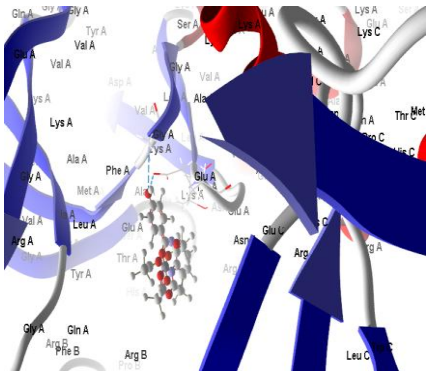


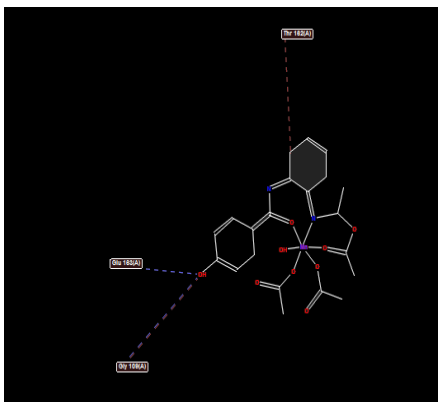
(A)





(B)





(C)

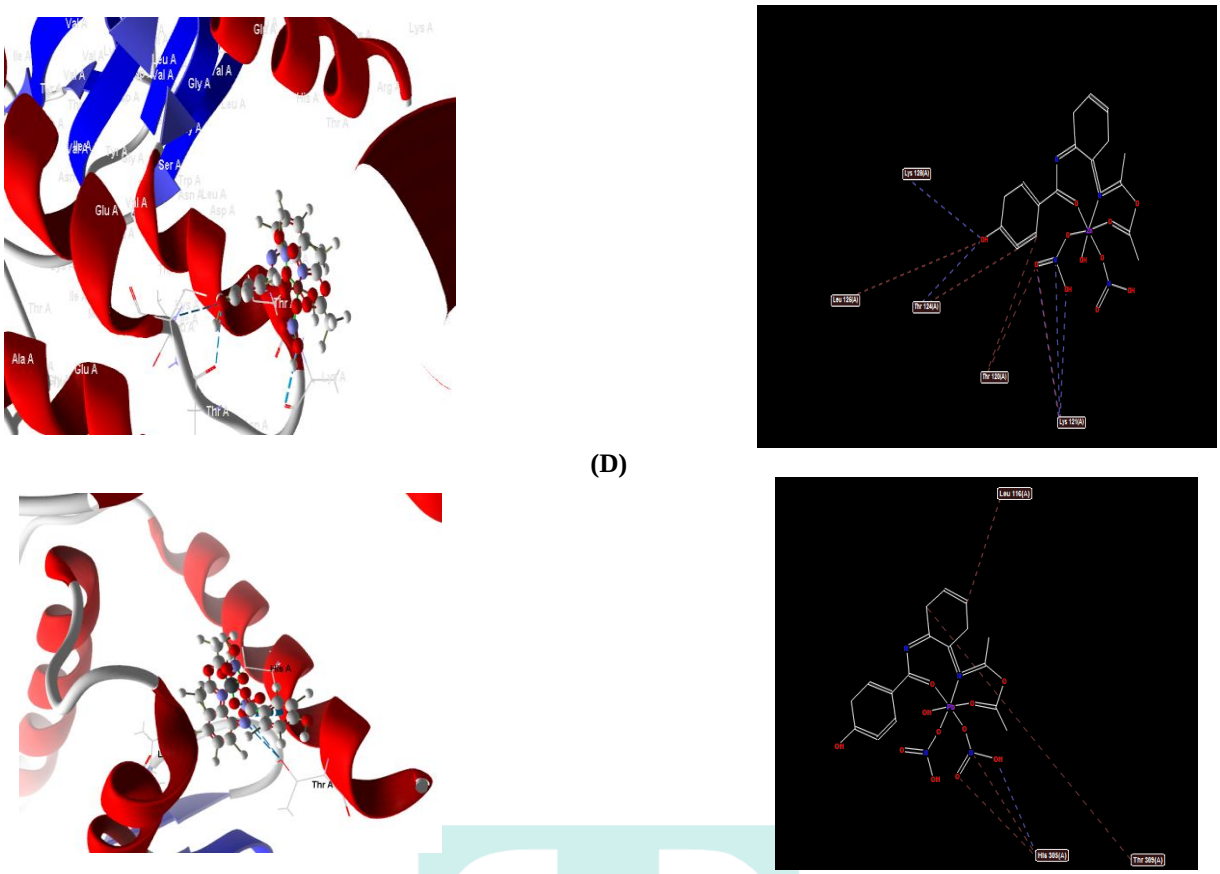


Fig. 5 Virtual Molecular docking and the 2D structure of the docked: **(A)** complex **(1)**, **(B)** complex **(2), (C)** complex **(3), (D)** complex **(4)** and **(E)** complex **(5)** with 2x7x

Biological studies "*In vivo* studies

Biochemical analyses

The results of the present study [Table 2] 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)	110.254±15.021	107.63±21.152	116.012±10.961	114.58±13.327	109.127±15.11	124.129±16.14
ALT (U/l)	41.327±3.208	38.655±4.958	42.880±5.983	36.639±6.871	40.987±8.160	44.117±5.525
ALP (U/l)	166.270±16.681	154.331±10.697	155.989±16.921	161.983±18.11	168.396±14.47	171.71±22.144
Alb (g/dl)	4.711 ±0.879	4.817 ±0.586	4.752 ±0.899	4.585±0.449	4.981±0.801	4.549±0.259
T. Bilirubin (mg/dl)	0.521±0.0981	0.4669±0.128	0.498±0.059	0.499±0.058	0.501±0.098	0.462±0.110
B. Urea (mg/dl)	35.624±4.608	33.691±3.351	39.221±6.241	34.881±4.308	30.661±3.997	37.417±4.528
S. Creatinine (mg/dl)	0.592 ±0.052	0.504 ±0.098	0.559 ±0.140	0.522±0.057	0.600±0.107	0.511±0.091

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)	16.921 ±2.996	16.581±1.689	16.228±3.781	16.891±2.986	16.147±2.221	17.414±3.448
RBCs (X 10^6/cmm)	7.101 ±0.881	6.898±0.696	7.056±1.211	6.622±1.527	6.01±0.571	5.991±1.051
TLC (X 10^3/cmm)	10.551±1.892	9.772±2.04	8.989±0.992	9.49±1.209	11.447±2.085	9.324±1.482
PLTs (X 10^3/cmm)	771.121±63.98	699.631±80.15	700.142±55.751	744.257±39.92	801.44±77.691	712.54±54.58

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 complexes NPs against human colorectal cancer cell line (HCT-116 cells), *invitro* study. Results showed potent effect of Mg and Pb complexes NPs and with lower degree in case of Cu, Mn and Zn complexes NPs in comparison with standard drug (Cisplatin) in a dose-dependent manner. 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 [37]. This result proving that the tested complexes NPs inhibit cell proliferation via induction of apoptotic cell death. In addition, the complexes 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 they have potential anticancer properties and can be applied as anti-cancer therapeutics. A previous study showed that metal complexes NPs' uptake by the cells endocytosis and emphasized intracellular release of the metal ions from the complex NPs (that) blocks cell division by binding to DNA causing DNA damage and contributed to the cytotoxicity and metabolic stress activates cell death via apoptosis. Also, down regulation of proliferating cell nuclear antigen, a factor critical for DNA replication and repair following metal NP treatment, supports the anti-proliferative effects of these nanoparticles [38]. 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 [39].

CONCLUSION

Reports indicate that metal complexes NPs might be useful as therapeutics in cancer therapy, and in combination with Hadron therapy led to an enhancement of strongly lethal DNA damage caused by double-strand breaks. A previous study showed that metal complexes NPs uptake by the cells endocytosis and emphasized intracellular release of M^{2+} blocks cell division by binding to DNA causing DNA damage and contributed to the cytotoxicity and metabolic stress activating cell death via apoptosis [40]. The metal complexes 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 [41]. Also, down regulation of proliferating cell nuclear antigen, a factor critical for DNA replication and repair following metal NPs treatment, supports the anti-proliferative effects of the metal complexes NPs [42]. The tested complexes NPs were thought to serve as a reservoir for metal ions that can induce DNA damage in cancer cells.

AUTHOR'S CONTRIBUTIONS

ASE designed the study and performed the complexes synthesis. AMA, AAD and EHM performed most of the experiments (Biochemical and hematological analyses), analyzed and interpreted the data. SAK performed and interpreted the molecular docking studies. AMA and EHM 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/9/23).

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