



## Nano-organometallic complexes as therapeutic platforms against breast cancer cell lines; (*in vitro* study)

Abdou S. El-Tabl<sup>1</sup>, Moshira M. Abdel Wahed<sup>2</sup>, Marwa M .El assaly<sup>1</sup> and Ahmed M.Ashour<sup>1</sup>



CrossMark

<sup>1</sup>Department of chemistry, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt

<sup>2</sup>Pathology Department, Faculty of Medicine, Menoufia University, Shebin El- Kom, Egypt

### Abstract

Improvements have been made to chemotherapies because drugs are still not reaching the tumor site at effective doses and are often associated with high systemic toxicities and poor pharmacokinetics. The nanotechnology allows more effective and less toxic chemotherapy. It has been shown that, many anticancer drugs are not able to penetrate more than 40-50 nm (equivalent to combined diameter of 3-5 cells from the vasculature). These defects lead to incomplete tumor response, multiple drug resistance and therapeutic failure. The best way to increase the efficacy and reduce the toxicity of a cancer drug is to direct the drug to its target and maintain its concentration at the site for a sufficient time for therapeutic action to take effect. Cu(II), Zn(II) and mixed Cu(II)/Zn(II) nano complexes center on opportunities for improving this process. Nano complexes of bioactive ligands had been prepared and spectroscopically characterized. The electron microscopic data confirmed the nanof orm of these complexes in the range (12.2 – 85.0 nm). Invitro antitumor activity of the complexes had been studied against breast cancer cell lines and the IC<sub>50</sub> values were detected to show that the order of the cytotoxic effect was complex (4) (Cu(II) acetate) > complex (1) (Cu(II) chloride) > complex (2) (mixed Cu(II)/Zn(II) acetate) > complex (3), (Zn(II) acetate). The invivo cytotoxicity of the complexes showed that, the complexes have no side effects after six weeks was confirmed by clinical and histopathological studies. The results augur well for breast cancer treatment.

**Keywords:** Bioactive ligands, nano complexes, spectral studies, invitro and invivo cytotoxicity.

### 1. Introduction

Breast cancer is still the leading cause of cancer deaths among women worldwide. It is the most frequent carcinoma in females and the second most common cause of cancer-related mortality in women. Approximately 61,000 new cases of in situ and 246,000 cases of invasive breast carcinoma, respectively, are expected to be diagnosed in the United States in 2016. Within this same period in the United States, breast cancer will account for an estimated 40,500 deaths among women [1]. Medicinal inorganic chemistry can exploit the unique properties of metal ions for the design of new drugs. This has, for instance led to the clinical application of chemotherapeutic agents for cancer treatment, such cisplatin; the use of cisplatin is however limited due to its toxic effects. This has spurred chemists to employ different strategies in the development of new metal-based anticancer agents with different mechanisms of

action [2]. The serendipitously found anticancer drug cisplatin and its second-generation congener carboplatin appear to be promising drug systems for the treatment of breast tumors, in particular of multidrug resistant and highly aggressive triple negative subtypes [3]. In the wake of these platinum drugs, complexes of the coinage metal copper, silver and gold were developed that showed enhanced selectivity for breast cancer while causing fewer and weaker side-effects [4]. International Organization for Standardization (ISO) defined a nanoparticle as a discrete Nano-object where all three Cartesian dimensions are less than 100 nm [5]. There are three major physical properties of nanoparticles, and all are interrelated: (1) they are highly mobile in the free state (2) they have enormous specific surface areas and (3) they have a vast range of compositions, depending on the use or the product [6]. Nanoparticles cannot only circulate widely throughout the body but also enter

\*Corresponding author e-mail: [ahmad\\_ashour81@yahoo.com](mailto:ahmad_ashour81@yahoo.com).

Receive Date: 11 October 2020, Revise Date: 25 November 2020, Accept Date: 30 November 2020

DOI: 10.21608/EJCHEM.2020.45020.2937

©2021 National Information and Documentation Center (NIDOC)

cells or be designed to bind specific cells [7]. Those properties had enabled new ways of enhancing images of organs as well as tumors and other diseased tissues in the body. Also, they had facilitated the development of new methods of delivering therapy, such as by providing local heating by blocking vasculature to diseased tissues and tumors [8]. This manuscript includes studying the spectroscopic characterization of some nano-metallic complexes of bioactive ligands, *Invitro* cytotoxic activity against breast cancer cell lines MCF-7 and also the *invivo* cytotoxic effect of these nanometric complexes against normal experimental animals.

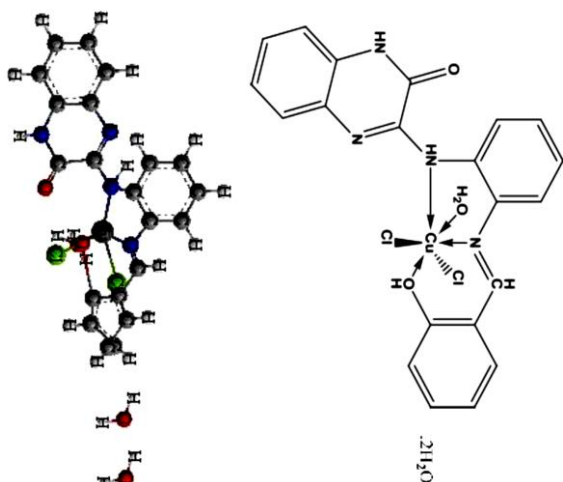
## 2. Materials and Methods

### 2.1. Chemicals and Instrumentations

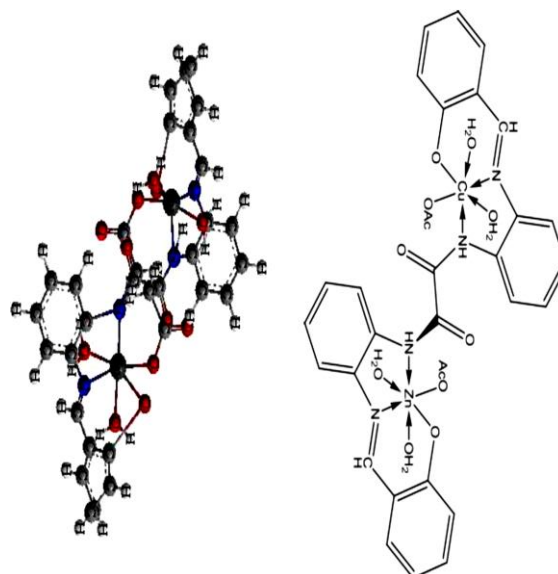
Four different metal complexes of bioactive ligands had been prepared, spectroscopically characterized and purified according to Abdou S. El-Tabl et al, in a previously published articles [9,10]. All solvents and reagents were of analytical grade. The analytical and physical data of the metal complexes are as follows:

**Table 1.** Analytical and physical data of metal complexes.

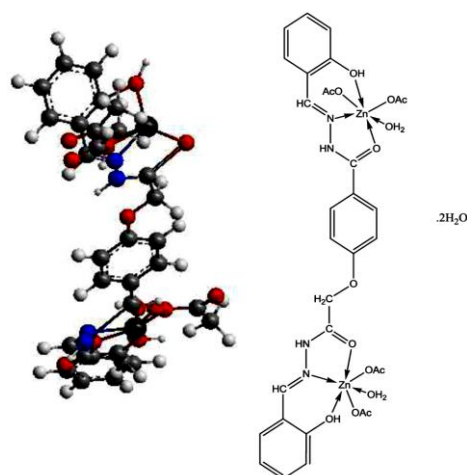
Complexes	Molecular Formula	Color	Molecular weight	Melting point
Complex (1)	$C_{21}H_{22}N_4O_5Cl_2Cu$	Black	544.88	> 300
Complex (2)	$C_{32}H_{34}N_4O_{12}CuZn$	Green	795.56	> 300
Complex (3)	$C_{25}H_{28}N_4O_9Zn_2$	Brown	832.08	> 300
Complex (4)	$C_{35}H_{36}N_4O_{15}Cu_2$	Black	830.08	> 300



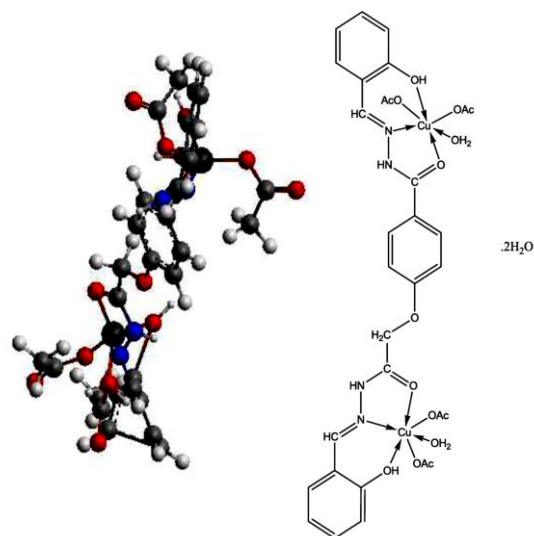
**Figure 1.** Structure of Cu(II) chloride complex



**Figure 2.** Structure of Cu(II)/Zn(II) acetate complex



**Figure 3.** Structure of Zn(II) acetate complex



**Figure 4.** Structure of Cu(II) acetate complex

## 2.2. Transmission electron microscopy 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 allowing the liquid carrier to evaporate in air then assaying by a JEOL 1230 transmission electron microscope (120 kV) [11].

## 2.3. In vitro studies:

Human breast cancer cell line (MCF-7) was purchased from (VACSRA), Giza-Egypt. Cells were maintained in complete growth medium, Roswell Park Memorial Institute medium (RPMI). [RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 U/ml)/streptomycin (100 µg/ml)]. The chemotherapeutic effect was measured in vitro for the synthesized complexes using the Sulfo-Rhodamine-B-stain (SRB) assay. Cells were plated in 96-multiwell plate (104 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compounds in DMSO under test (0, 1.56, 3.125, 6.5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37°C and under atmosphere of 5% CO<sub>2</sub>. After 48 hours, cells were fixed, washed and stained with Sulfo-Rhodamine-B-stain. Excess stain was washed with acetic acid and attached stain was recovered with *Tris* EDTA buffer (10 mM Tris HCl, 1 mM disodium EDTA, pH=7.5-8). Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified compound. All culture reagents were obtained from (Lonza) supplier, Egypt. Sulfo-Rhodamine-B-stain (SRB) assay was performed following the protocols of Skehan, P., R. Storeng, et al. to evaluate the cytotoxicity in MCF-7 cells [12].

## 2.4. In vivo studies

### 2.4.1. Animals

90 healthy male albino rats 8 weeks old (180 - 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.

### 2.4.2. Acute toxicity study

Determination of lethal dose 50 (LD<sub>50</sub>) using experimental animals. In screening drugs, determination of LD<sub>50</sub> is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. The LD<sub>50</sub> of the studied compounds was determined as described by Akhila et al. [13]. The acute intraperitoneal toxicity of the chosen complexes was done on 40 animals (10 per group). The 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 injected intraperitoneal. The chosen complexes were administered with graded doses of 1×10<sup>-6</sup>, 5×10<sup>-6</sup>, 1×10<sup>-5</sup> and reached to 1×10<sup>-4</sup> mmole/L/Kg body weight under the same environmental conditions. After administration of the chosen complexes 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<sub>50</sub>). The LD<sub>50</sub> value of the complexes was determined. The LD<sub>50</sub> for all tested complexes nanoparticles were devoid of any toxicity in rats when given the selected different doses by intraperitoneal route.

### 2.4.3. Experimental design

Animals were allowed 10 days for adaptation. They were then randomly distributed into 5 equal groups, 10 rats each. The animal groups were recognized as follows:

1. Group 1 (Control): Normal healthy control animals.
2. Group 2: Each animal was injected intra peritoneal with 1×10<sup>-5</sup> mmole/L of complex (1) for 6 weeks.
3. Group 3: Each animal was injected intra peritoneal with 1×10<sup>-5</sup> mmole/L of complex (2) for 6 weeks.
4. Group 4: Each animal was injected intra peritoneal with 1×10<sup>-5</sup> mmole/L of complex (3) for 6 weeks.
5. Group 5: Each animal was injected intra peritoneal with 1×10<sup>-5</sup> mmole/L of complex (4) for 6 weeks.

### 2.4.4. Blood collection

At the end of the experimental period (6 weeks), blood samples were collected from overnight rats, centrifuged at 3000 rpm for 10 min and the separated sera were frozen at -20 °C for future biochemical analysis.

### 2.4.5. Biochemical analyses

Liver enzymes activities, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using kinetic kits purchased by Human Diagnostic Kits, Germany [14]. The liver

function, albumin concentration and kidney functions, blood urea and serum creatinine were measured using Diamond Diagnostic kits, Egypt [15]. All biochemical analysis were determined using a Biosystems BTS-310 Spectrophotometer.

#### 2.4.6. Hematological analyses

Determination of hemoglobin (Hb) using Drabkin's solution [16], red blood corpuscles count (RBCs), total leucocytic count (TLC) and platelets count (PLTs) were determined manually [17,18].

### 2.5. 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 16.00 software. The results were expressed as mean  $\pm$  SE and the differences were considered significant at  $P \leq 0.05$ .

## 3. Results and discussions

### 3.1. Transmission electron microscopy characterization (TEM)

The average diameter of the all four complexes particles was determined to be  $29.02 \pm 5.02$  nm,  $34.88 \pm 7.84$  nm,  $38.42 \pm 20.01$  nm and  $25.82 \pm 7.40$  nm respectively as shown in figures 5 - 8

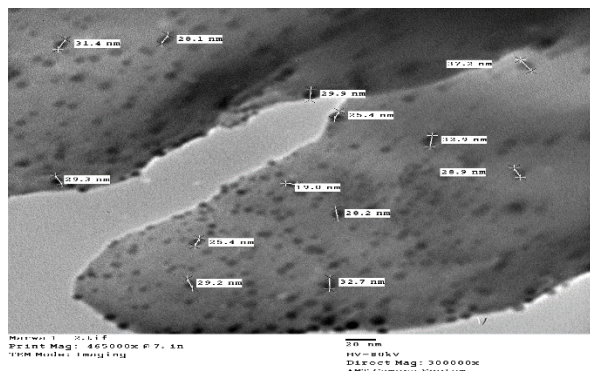


Figure 5. TEM images for Cu(II) chloride complex

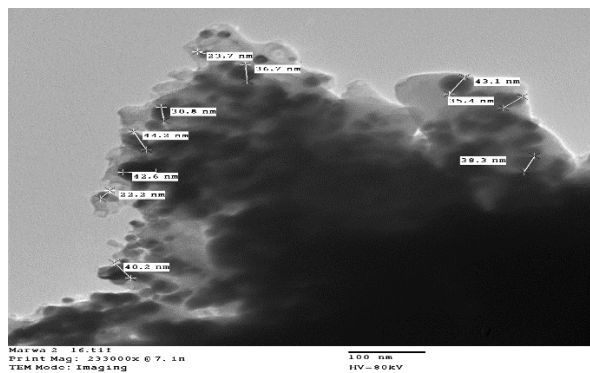


Figure 6. TEM images for Cu(II)/Zn(II) acetate complex

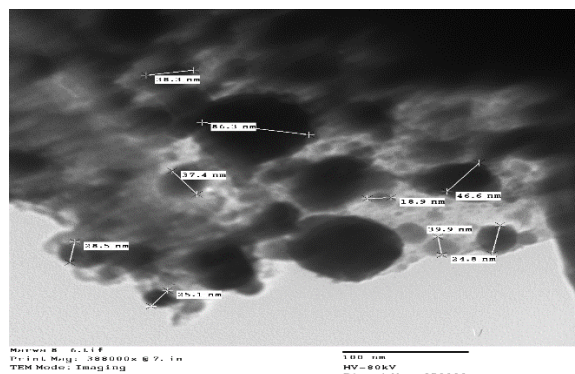


Figure 7. TEM images for Zn(II) acetate complex

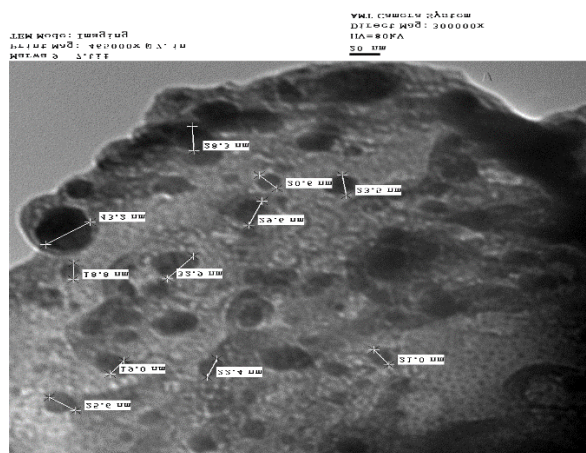


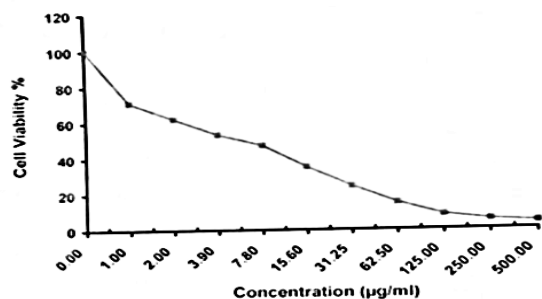
Figure 8. TEM images for Cu(II) acetate complex

All complexes are present in nano size particles i.e., their particles present 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-nanoparticulate 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 [19].

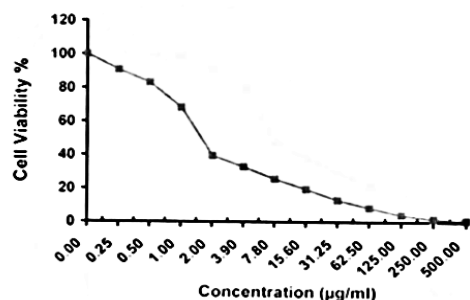
### 3.2. In vitro studies:

#### 3.2.1. Evaluation of the cytotoxic effect of different complexes on MCF-7 cell line by SRB assay:

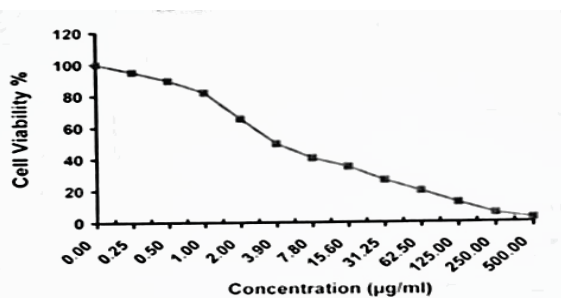
Cytotoxicity results indicated that the tested complexes NPs have  $IC_{50} = 1.63, 7.49, 20.3$  and  $1.42$   $\mu\text{g/ml}$  respectively demonstrated potent cytotoxicity against MCF-7 cancer cells whereas the  $IC_{50}$  of the standard drug cisplatin was  $5.71$   $\mu\text{g/ml}$ .



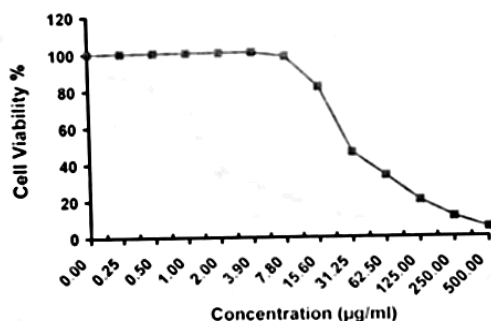
**Figure 9.** Mean IC<sub>50</sub> values of standard drug (Cisplatin) detected by Sulfo-Rhodamine-B-stain (SRB) assay on MCF-7 cell line.



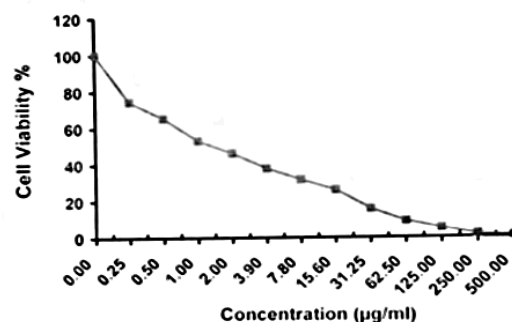
**Figure 10.** Mean IC<sub>50</sub> values of complex (1) (Cu (II) chloride ) detected by by Sulfo-Rhodamine-B-stain (SRB) assay on MCF-7 cell line.



**Figure 11.** Mean IC<sub>50</sub> values of complex (2) (mixed Cu(II)/Zn(II) acetate) detected by Sulfo-Rhodamine-B-stain (SRB) assay on MCF-7 cell line.

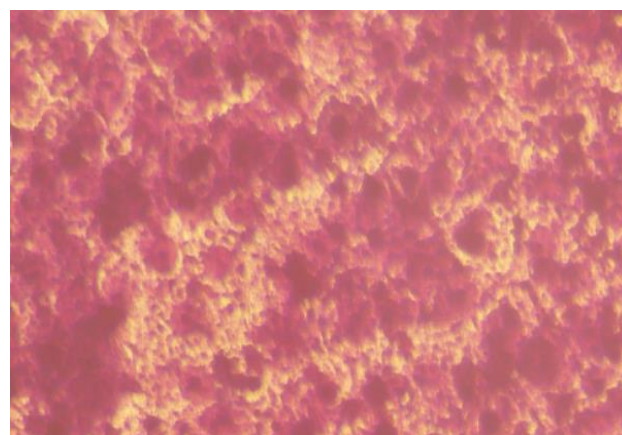


**Figure 12.** Mean IC<sub>50</sub> values of complex (3) (Cu(II) acetate) detected by by Sulfo-Rhodamine-B-stain (SRB) assay on MCF-7 cell line.

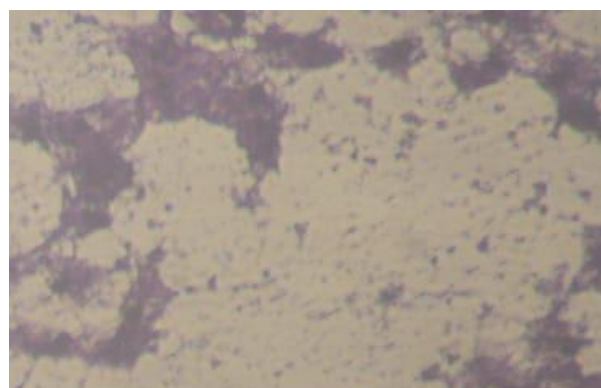


**Figure 13.** Mean IC<sub>50</sub> values of complex (4) (Zn(II) acetate) detected by by Sulfo-Rhodamine-B-stain (SRB) assay on MCF-7 cell line.

The microscopic histograms show the chemotherapeutic activity of the tested complexes NPs by comparing them with the standard drug (Cisplatin) as shown below. There is decreasing in the number of available cells. Most of the remaining observed degeneration changes in the form of irregular cell membrane opaque and not well formed chromatin regulated of swelling cytoplasm, other showed optatic change in the formed of chrunked cells and increase eosinophilia cells, and picknotic nucleus as shown in figures 14 - 26 .



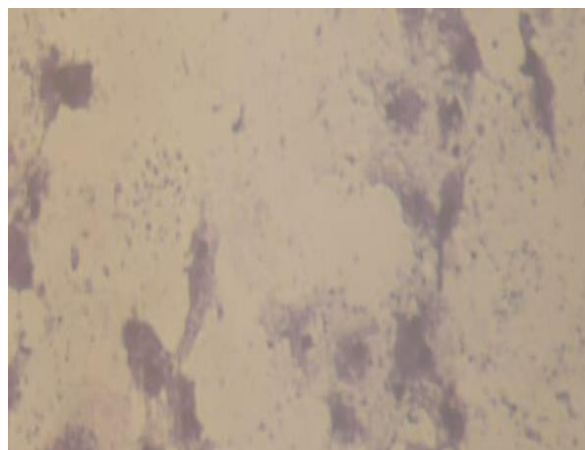
**Figure 14.** Histogram show non-treated MCF-7 cells



**Figure 15.** Histogram show MCF-7 cells treated with complex (1) nanoparticles at 0.5 µg/ml.



**Figure 16.** Histogram show MCF-7 cells treated with complex (1) nanoparticles at 100 µg/ml



**Figure 19.** Histogram show MCF-7 cells treated with complex (2) nanoparticles at 100 µg/ml



**Figure 17.** Histogram show MCF-7 cells treated with complex (1) nanoparticles at 500 µg/ml



**Figure 20.** Histogram show MCF-7 cells treated with complex (2) nanoparticles at 500 µg/ml



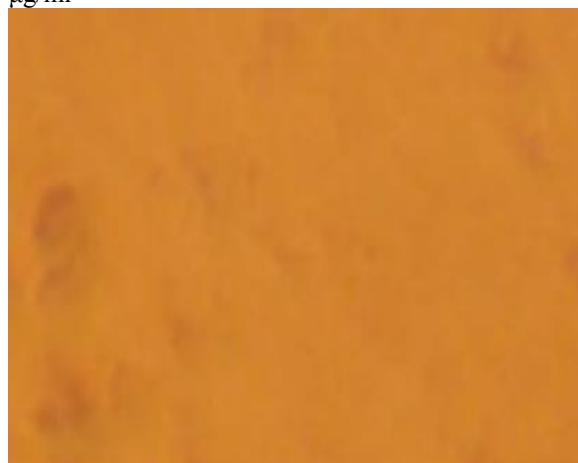
**Figure 18.** Histogram show MCF-7 cells treated with complex (2) nanoparticles at 0.5 µg/ml



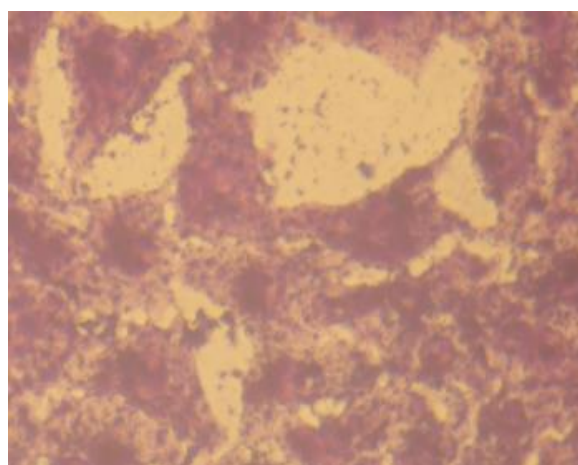
**Figure 21.** Histogram show MCF-7 cells treated with complex (3) nanoparticles at 0.5 µg/ml



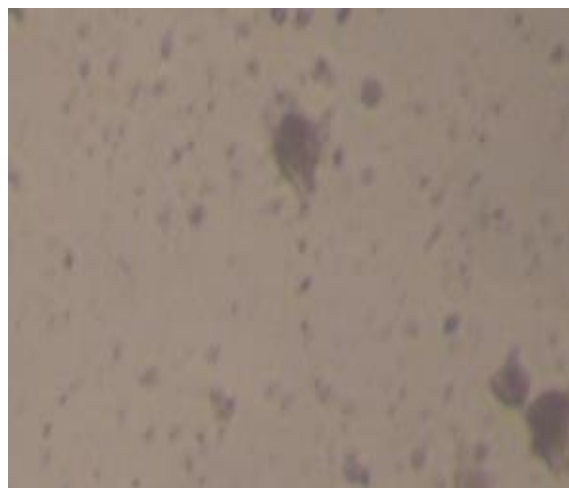
**Figure 22.** Histogram show Histogram show MCF-7 cells treated with complex (3) nanoparticles at 100 µg/ml



**Figure 23.** Histogram show Histogram show MCF-7 cells treated with complex (3) nanoparticles at 500 µg/ml



**Figure 24.** Histogram show MCF-7 cells treated with complex (4) nanoparticles at 0.5 µg/ml



**Figure 25.** Histogram show MCF-7 cells treated with complex (4) nanoparticles at 100 µg/ml



**Figure 26.** Histogram show MCF-7 cells treated with complex (4) nanoparticles at 500 µg/ml

The obtained data indicate the surviving fraction ratio against MCF-7 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 complexes may be attributed to the central metal atom which was explained by Tweedy's chelation theory [20, 21]. 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 [22]. Moreover, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction [23].

### 3.3. In vivo studies

#### Biochemical analyses

The results of the present study [Table 1] recorded that the liver functions (AST, ALT, and albumin)

measured in the serum show no significant differences between treated groups and the control group. Also there are no any significant differences in renal functions [Table 2] or hematological parameters [Table 3] between all tested groups.

**Table 2.** Statistical analysis (ANOVA) for liver function tests in the different groups

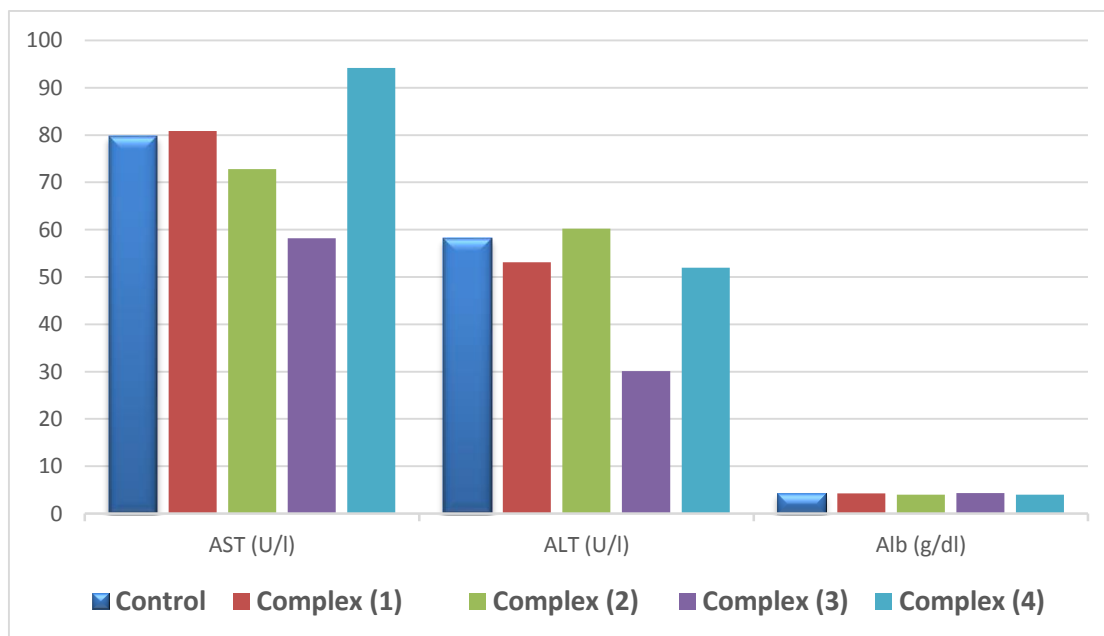
Parameters	Control	Complex (1)	Complex (2)	Complex (3)	Complex (4)
AST (U/l)	79.67±4.91 <sup>ab</sup>	80.85±6.19 <sup>ab</sup>	72.820±5.11 <sup>c</sup>	58.191±5.97 <sup>d</sup>	94.21±7.309 <sup>e</sup>
ALT (U/l)	58.11±3.547 <sup>ac</sup>	53.10±4.291 <sup>bc</sup>	60.22±6.27 <sup>c</sup>	30.112±2.940 <sup>d</sup>	51.96±3.941 <sup>bc</sup>
Alb (g/dl)	4.29±1.124 <sup>abd</sup>	4.27 ±1.09 <sup>abd</sup>	3.99 ±1.05 <sup>ce</sup>	4.31 ±1.05 <sup>abd</sup>	4.02 ±1.05 <sup>ce</sup>

ANOVA: analysis of variance, SD: standard deviation; each value is represented as mean ± SD. Data with different superscripts are significantly different at  $p \leq 0.05$ , <sup>a</sup>Significance versus control group, <sup>b</sup>Significance versus group treated with complex (1) with  $1 \times 10^{-5}$  mmole/L, <sup>c</sup>Significance versus group treated with complex (2) with  $1 \times 10^{-5}$  mmole/L, <sup>d</sup>Significance versus group treated with complex (3) with  $1 \times 10^{-5}$  mmole/L, <sup>e</sup>Significance versus group treated with complex (4) with  $1 \times 10^{-5}$  mmole/L.

**Table 3.** Statistical analysis (ANOVA) for renal function tests in the different groups

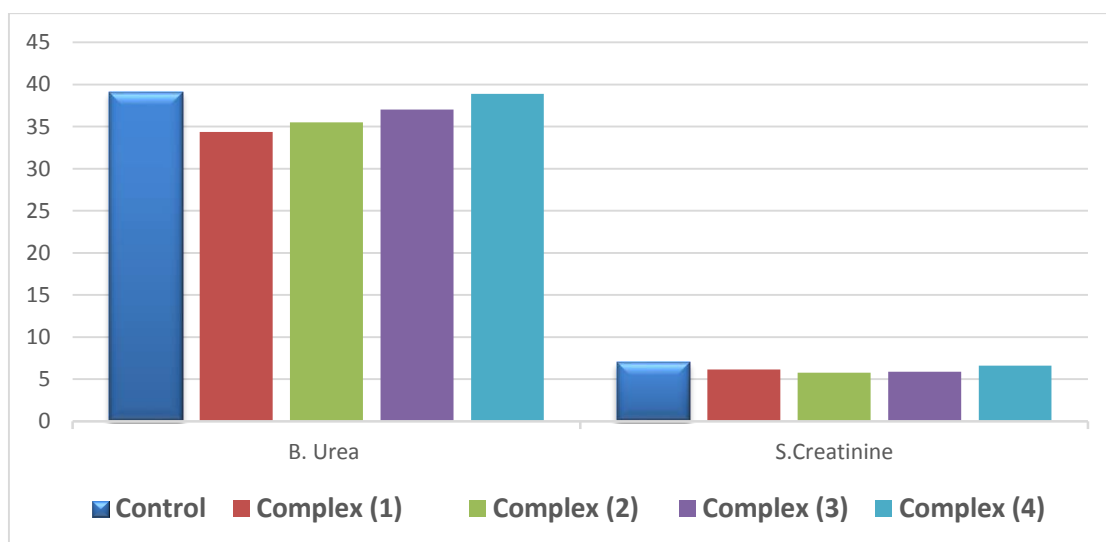
Parameters	Control	Complex (1)	Complex (2)	Complex (3)	Complex (4)
B. Urea(U/l)	39.112±2.429 <sup>ae</sup>	34.374±2.911 <sup>bc</sup>	35.512±3.003 <sup>bc</sup>	37.047±2.681 <sup>d</sup>	38.913±2.115 <sup>ae</sup>
S.Creatinine	0.697±0.127 <sup>ae</sup>	0.617±0.110 <sup>b</sup>	0.576±0.092 <sup>cd</sup>	0.588±0.166 <sup>cd</sup>	0.66±0.126 <sup>ae</sup>

ANOVA: analysis of variance, SD: standard deviation; each value is represented as mean ± SD. Data with different superscripts are significantly different at  $p \leq 0.05$ , <sup>a</sup>Significance versus control group, <sup>b</sup>Significance versus group treated with complex (1) with  $1 \times 10^{-5}$  mmole/L, <sup>c</sup>Significance versus group treated with complex (2) with  $1 \times 10^{-5}$  mmole/L, <sup>d</sup>Significance versus group treated with complex (3) with  $1 \times 10^{-5}$  mmole/L, <sup>e</sup>Significance versus group treated with complex (4) with  $1 \times 10^{-5}$  mmole/L.

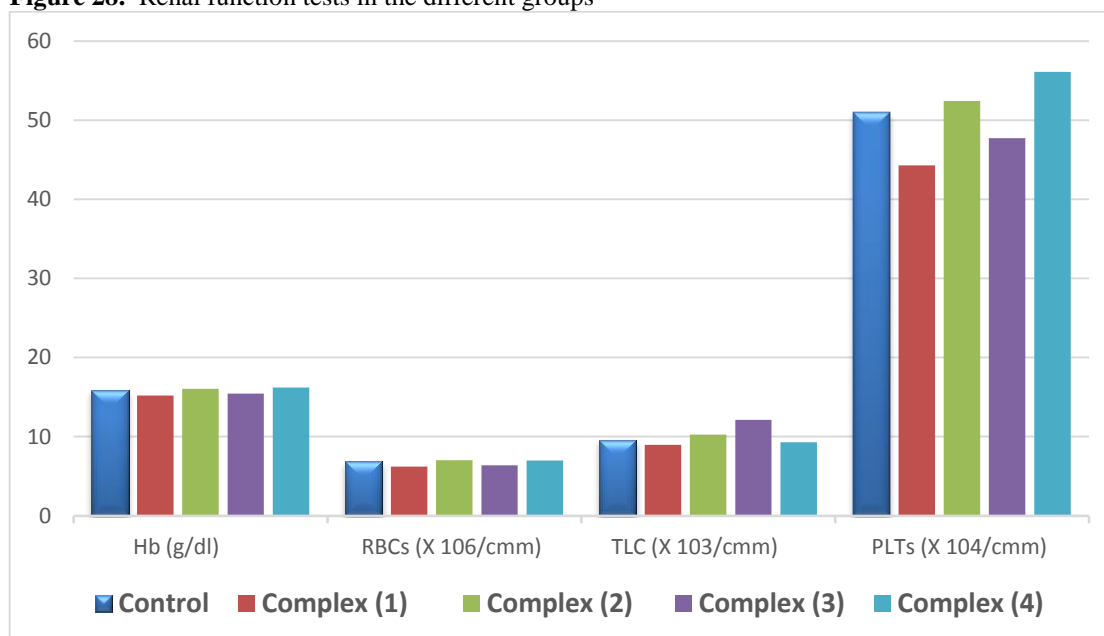


**Figure 27.** Liver function tests in the different groups





**Figure 28.** Renal function tests in the different groups



**Figure (29).** Hematological tests in the different groups

**Table 4.** Statistical analysis (ANOVA) for hematological tests in the different groups

Parameters	Control	Complex (1)	Complex (2)	Complex (3)	Complex (4)
<b>Hb (g/dl)</b>	15.91 ±2.27 <sup>ac</sup>	15.210±1.17 <sup>bd</sup>	16.04±3.07 <sup>ac</sup>	15.421±2.911 <sup>bd</sup>	16.21±1.77 <sup>e</sup>
<b>RBCs (X 10<sup>6</sup>/cmm)</b>	6.84 ±1.14 <sup>ace</sup>	6.221±1.98 <sup>bd</sup>	7.017±1.257 <sup>ace</sup>	6.39 ±0.994 <sup>bd</sup>	6.99±2.006 <sup>ace</sup>
<b>TLC (X 10<sup>3</sup>/cmm)</b>	9.61±2.062 <sup>ae</sup>	8.96 ±1.598 <sup>b</sup>	10.27±1.087 <sup>c</sup>	12.14±3.102 <sup>d</sup>	9.29±1.633 <sup>ae</sup>
<b>PLTs (X 10<sup>4</sup>/cmm)</b>	50.98±41.12 <sup>ac</sup>	44.299±30.14 <sup>bd</sup>	52.416±28.978 <sup>ac</sup>	47.711±31.22 <sup>bd</sup>	56.111±36.11 <sup>ac</sup>

ANOVA: analysis of variance, SD: standard deviation; each value is represented as mean ± SD. Data with different superscripts are significantly different at  $p \leq 0.05$ , <sup>a</sup>Significance versus control group, <sup>b</sup>Significance versus group treated with complex (1) with  $1 \times 10^{-5}$  mmole/L, <sup>c</sup>Significance versus group treated with complex (2) with  $1 \times 10^{-5}$  mmole/L, <sup>d</sup>Significance versus group treated with complex (3) with  $1 \times 10^{-5}$  mmole/L, <sup>e</sup>Significance versus group treated with complex (4) with  $1 \times 10^{-5}$  mmole/L.

#### 4. Conclusion

Reports indicate that some metal complexes NPs might be useful as therapeutics in cancer therapy. All tested complexes NPs led to an enhancement of strongly lethal DNA damage caused by double-strand break [24]. Accordingly, the cytotoxic effects and the biological activity of the complexes NPs as antitumor agents were examined in vitro against human breast cancer cell line (MCF-7 cells) using Sulfo-Rhodamine-B-stain (SRB) assay. Results showed potent effect of the complexes NPs more by comparison with standard drug (Cisplatin) in a dose-dependent manner, where increasing concentration of both of them resulted in increased percentage of dead cells [25]. In addition, 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 cancer therapeutics [26]. A previous study showed that Cu complex NPs uptake by the cells endocytosis and emphasized intracellular release of  $\text{Cu}^{2+}$  blocks cell division by binding to DNA causing DNA damage and contributed to the cytotoxicity and metabolic stress activating cell death via apoptosis [27]. 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 [28]. All tested complexes NPs are thought to serve as a reservoir for metal ions that can induce DNA damage in cancer cells [29].

#### References

- Pieter C.A.Bruijninx and Peter J.sadler: New trends for metal complexes with anticancer activity. *Current opinion in chemical Biology*; 12(2) 197-206 (2008).
- Ali I, Wani WA, Saleem K, et al. Platinum compounds: a hope for future cancer chemotherapy. *Anticancer Agents Med Chem*; 13: 296–306 (2013).
- B.Biersack,A.ahmed,F.H.Sarkar and R.Schobert: Coinage Metal Complexes Against Breast Cancer. *Current medicinal chemistry*; 19(23), 3949-3956 (2012).
- Shiow Jin Tan, Yaw Kai Yan, Peter Peng Foo Lee and Kok Hwa Lim: Copper, gold and silver compounds as potential new anti-tumor metallodrugs. *Future medicinal chemistry*; VOL.2 (2010).
- R. Mishra, J. Militky,V. Baheti, J. Huang,B. Kale,M. Venkataraman,V. Bele,V. Arumugam,G. Zhu and Y. Wang: The production, characterization and applications of nanoparticles in the textile industry. *Journal of Textile Progress*; vol. 46 (2014).
- Mario M. Modena, Bastian Rühle Thomas, P. Burg Stefan Wuttke: Nanoparticle Characterization: What to Measure?. Wiley on line Library; (2019).
- Jesús Mosquera , Isabel García, and Luis M. Liz-Marzán: Cellular Uptake of Nanoparticles versus Small Molecules, A Matter of Size. *Accounts of Chemical Research*; 51, 9, 2305–2313 (2018).
- Marian E Gindy &Robert K Prud'homme: Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. *Expert Opinion on Drug Delivery*; Vol. 6 (2009).
- Saad El-Tabl, A., M. Abd-Elwahed, et al. Synthesis, spectral characterisation and cytotoxic effect of metal complexes of 2-(2-(4-carboxyphenyl)guanidino) acetic acid ligand. *Chemical Speciation & Bioavailability*; 25(2): 133-146 (2013).
- AS El-Tabl, MT Shaban, NM Abd El-Wahed: Novel Metal Complexes as Antimicrobial Agents, Synthesis and Spectroscopic Characterization. *Journal of Chemistry and Chemical Sciences*; Vol. 9(3), 74-108 (2019).
- Vahid Samaeeaghmiyonia, Hosni Idrissi, Jonas Groten, Ruth Schwaiger, Dominique Schryvers: Quantitative in-situ TEM nanotensile testing of single crystal Ni facilitated by a new sample preparation approach. *Micron*;Vol. 94, 66-73 (2017).
- SamirY.Abbas, KhairyA.M.El-Bayouki, Wahid M.Basyouni & Eslam A. Mostafa: New series of 4(3H)-quinazolinone derivatives: syntheses and evaluation of antitumor and antiviral activities. *Medicinal Chemistry Research*; Vol. 27, 571–582 (2018).
- Akhila JS, Deepa S and Alwar MC. Acute toxicity studies and determination of median lethal dose. *Current Science*; 93: 917–920 (2007).
- Tietz NW, Rinker AD, Shaw LM: IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. *Journal of clinical chemistry and clinical biochemistry.*; 21:731-748. (1983).
- Wilson, K. and Walker, J: Practical Biochemistry: Principles and Techniques, Cambridge University Press. (2000).
- Mazzela F.M., Schumacher H.R: Hemoglobin. *Clinical Chemistry. Theory. Analysis and Correlation*, 40: 771-789. (2010).
- NCCLS document H18-A: Procedures for the handling and processing of blood specimens. National Committee for Clinical Laboratory Standards, Villanova, PA. (1990).
- Rutherford, N.J, Becton, Dickinson: Becton-Dickinson. Unopette WBC/Platelet determination for manual methods. Rutherford, N.J.: Becton, Dickinson, and Company. (1996).

19. Eunkeu Oh, James B. Delehanty, Kim E. Sapsford et al: Cellular Uptake and Fate of PEGylated Gold Nanoparticles Is Dependent on Both Cell-Penetration Peptides and Particle Size. *ACS Nano*; 5, 8, 6434–6448. (2011).
20. Illán-Cabeza, N.A., A.R. García-García, et al: Synthesis, characterization and antiproliferative behavior of tricarbonyl complexes of rhenium (I) with some 6-amino-5-nitrosouracil derivatives: Crystal structure of fac-[ReCl(CO)<sub>3</sub>(DANU-N5, O4)](DANU= 6-amino-1, 3-dimethyl-5-nitrosouracil). *Journal of inorganic biochemistry*.; 99(8): 1637-1645. (2005).
21. Saad El-Tabl, A., M. Abd-Elwahed, et al: Synthesis, spectral characterisation and cytotoxic effect of metal complexes of 2-(2-(4-carboxyphenyl) guanidino) acetic acid ligand. *Chemical Speciation and Bioavailability*.; 25(2): 133-146 (2013).
22. Feng, G., J. C. Mareque-Rivas, et al: A highly reactive mononuclear Zn (II) complex for phosphodiester cleavage. *Journal of the American chemical society*.; 127(39): 13470-13471 (2005).
23. M. Jarenmark, E. Martin, et al: Unsymmetrical dizinc complexes as models for the active sites of phosphohydrolases. *Dalton Transactions*, vol. 39, pp. 8183-8194 (2010).
24. Asharani PV, Xinyi N, Hande MP, et al: DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticles. *Nanomedicine (Lond)*.; 5(1): 51–64 (2010).
25. Amel F.M. Ismail, Mamdouh M. Ali, Laila F.M. Ismail et al: Photodynamic therapy mediated antiproliferative activity of some metal-doped ZnO nanoparticles in human liver adenocarcinoma HepG2 cells under UV irradiation. *Journal of Photochemistry and Photobiology B: Biology* Vol. 138, 99-108.(2014).
26. Abdou S. El-Tabl, Moshira M. Abd-El Wahed, Ahmed M. Ashour et al: Metallo-organic Copper(II) Complex in Nano Size as a New Smart Therapeutic Bomb for Hepatocellular Carcinoma. *Journal of Chemistry and Chemical Sciences*; Vol. 9(1), 33-44, (2019).
27. Grassi Perin & Mario Danilo: Biomaterials for biotechnological applications: synthesis and activity evaluations. Università degli studi di Trieste. (2010).
28. Muddasarul Huda, Sankar Pajaniradje et al: Anti-proliferative and apoptosis-triggering potential of disulfiram and disulfiram-loaded polysorbate 80-stabilized PLGA nanoparticles on hepatocellular carcinoma Hep3B cell line. *Nanomedicine: Nanotechnology, Biology and Medicine*; Vol. 12(6), 1641-1650 (2016).
29. Serguei Vinogradov, Galya Warren & Xin Wei: Macrophages associated with tumors as potential targets and therapeutic intermediates. *Nano medicine*; Vol. 9(5), (2014).