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In vitro assessments of cytotoxic and cytostatic effect of two [Cu(dien)(N-N)]Br_2 complexes on L6, HCT, PC3 and HepG2 cancer cells

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Abstract

Two new Cu(II) complexes of type [Cu(dien)(N-N)] Br_2 (dien is diethlylenetriamine, C1 complex with N-N = diethlylenetriamine and C2 complex with N-N = propyl-1,3-diamine) were made valuable and *in vitro* screened for both cytostatic and cytotoxic activities against four cell lines: colon cancer (HCT), liver cancer (HepG2), prostate cancer (PC3) and as control cell (L6) human muscle cell was used *via* MTT test. The results reflected these complexes as a promising activity antiprolifrative agent against the used cell lines indicating that C1 and C2 complexes have a high anticancer activity at non-toxic concentrations.

Keywords: Copper(II), Diamine, MTT, Anticancer.

1. Introduction

The first proof that complexes of copper may have antitumor effeteness was reported when thiosemicarbazones complexes (TSCs) were introduced to medicinal chemistry, which was reported in early 1960s reflected their anticancer activities [1-3]. TSCs were medically tested due to their DNA enzyme ribonucleotide diphosphate reductase inhibitions and their selectivity to the cancers hormone-responsive [4]. Copper(II)/diamine/X_2 complexes are considered to be vital class of complexes because of their applications in several science fields [5-10]. They can be considered as simple models to understand the medical behavior of Cu-proteins unit [11-16].

New Cu(II) complexes originated from N,N,N-tripodal ligand using diethlylenetriamine and N,N-diamines ligands have been synthesized [1-13]. Diverse types of triamine and diamines ligands were complexed to dicationic Cu(II) mononuclear ions with high yield and in facile one pot reaction [6]. These complexes were fully characterized by electrochemical, thermal, spectroscopic and XRD-ray crystallographic analysis [8]. The UV-Visible and XRD-ray diffraction data reflected a distorted trigonal bipyramidal geometry around Cu(II) ions with one solvated water molecule [4]. Theses complexes reflected a promising antibacterial results and anticancer activities were collected by testing these complexes against several cell lines and bacterial families [16].

Recently, we have prepared and developed many organic compounds containing S, O, N and P atoms in their backbones, such compounds have been tested as bioactivity drugs, anticorrosion and as ligands for coordination with several metal ions [17-27]. In this paper, we report the cytotoxic and cytostatic effect of [Cu(dipn)(N-N)]Br_2 (C1 and C2) complexes on selected cancer cell lines.

2. Methodology

2.1. Preparation of the complexes

Complex of [Cu(dipn)Br]Br and complexes (C1 and C2) the type [Cu(dipn)(N-N)]Br_2 dien is diethlylenetriamine and (N-N diamine) is ethylenediamine (C1) and propyl-1,3-diamine (C2) were prepared by the reaction of dien with $CuBr_2$ ions flowed by NN ligands in situ addition in [1:1:1] molar ratio using ethanol: water solution.

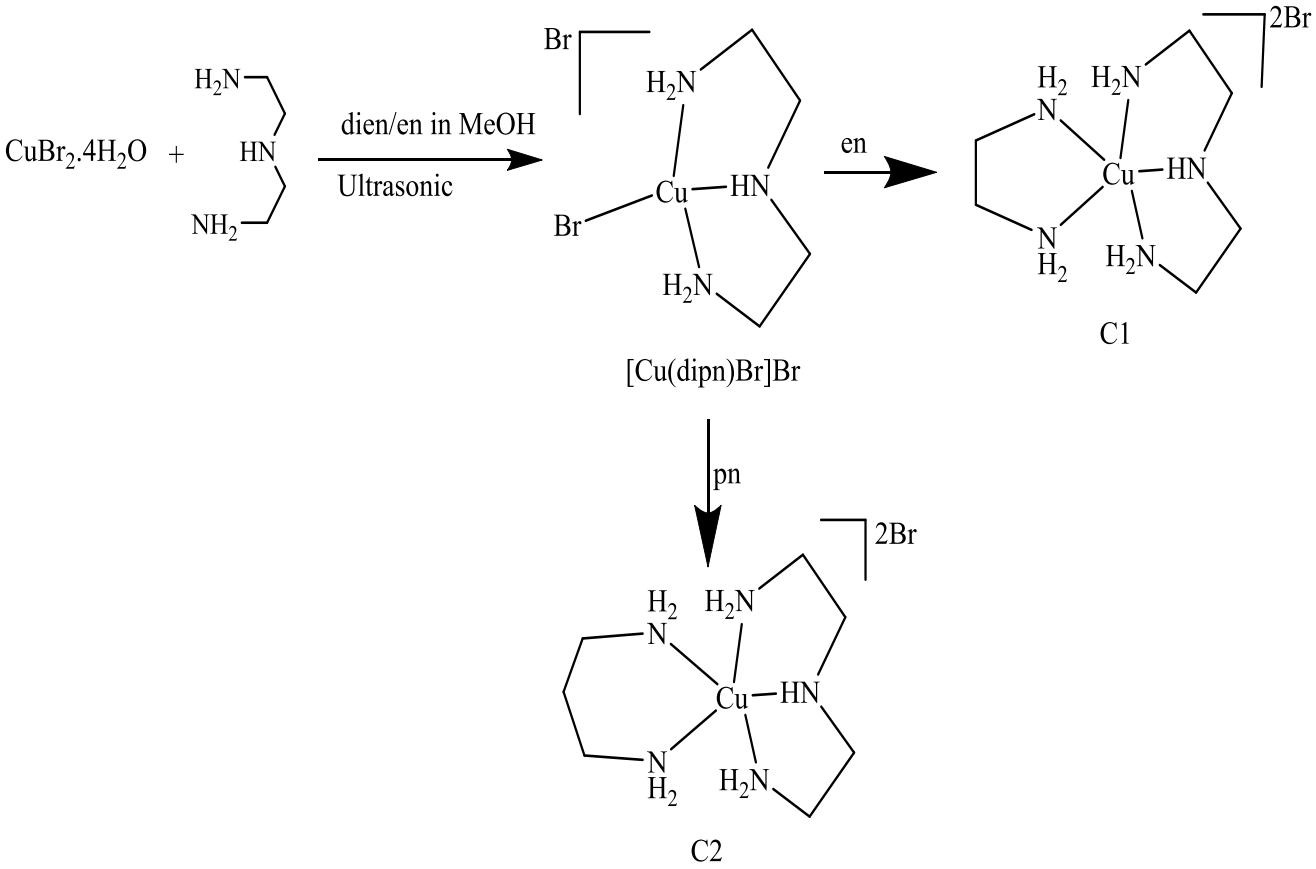
2.2 Stock solution, cell lines and MTT assay

All the stock solution together with RPMI media were prepared in absolute solvents [16], all the normal and human cancer cells were grown in Dulbecco's modified Eagle's medium (DMEM) [17]
The cytotoxicity MTT and cytostatic assays were performed using modified processer [17].

3. Results and discussion

3.1 Complexes

Under ultrasonic mode of radiation in ethanolic/water medium C1 and C2 copper(II) complexes have been isolated as bromide salts in good yields [4]. They have been characterized using elemental analysis and spectral methods [5]. Theses complexes are blue in color and are soluble in water (Scheme 1).



Scheme 1: Complexes synthesis.

3.2 *C1 and C2 cytotoxic effect*

Results showed that the starting material [Cu(dipn)Br]Br complex didn't induce cytotoxic effect at any concentration levels using all cancer cells type (used in this test), as shown in Figure 1. Cytotoxic experiments were performed with L6, Hct, PC3 and HepG2 cells using the starting [Cu(dipn)Br]Br complex in a humidified atmosphere, cells incubated with the [Cu(dipn)Br]Br complex for 24 hours, the lines represent the mean ± SD of three independent experiments carried out in triplicates.

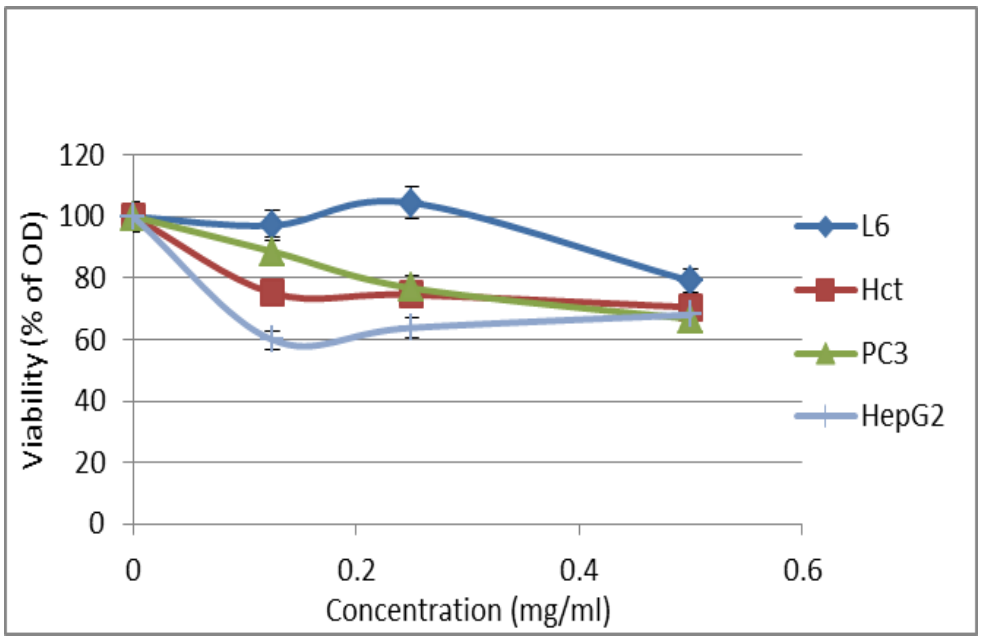


Fig.1. Cytotoxic with L6, HCT, PC3 and HepG2 cells using the [Cu(dipn)Br]Br copper.

Results show that C1 induced cytotoxic effect with L6 cells at very low concentration 0.03125 mg/ml. IC50 for the complexes were C1 0.07 and C2 0.18 as shown in Figure 3 and Table 1. *HCT cells*, C1 induced a cytotoxic effect at very low concentration 0.03125 mg/ml, meanwhile, C2 induced cytotoxicity at concentration higher than 0.5 mg/ml. Therefore, IC50 for C1 found to be 0.03, meanwhile for C2 found to be 0.3, as shown in Figure 2 and Table 1. Actually, C1 revealed promising antiproliferative effectiveness against the HCT cell lines with 0.03IC50, theses data are very promising when compared with the 0.07 IC50 value of the same complex on the normal cell line L6, which revealed C1 as high anticancer effeteness at nontoxic concentrations. *PC3 cells*, C1 induced cytotoxic effect at very low concentration 0.03125 mg/ml and C2 induced cytotoxicity at concentration higher than 0.5 mg/ml on the same cells with IC50 for C1 = 0.02 and for C2 = 0.17, as shown in Figure 2c and Table 1. C1 possess promising antiproliferative activity against PC3 cell line with 0.02 IC50 value, such values are promising when compared to the IC50 value of the same complex with 0.07 IC50 value on L6 cell which indicated that this complex has an anticancer effeteness at low concentrations. *HepG2 cells*, C1 induced cytotoxic effect at very low concentration 0.03125 mg/ml and C2 induced cytotoxicity at concentrations higher than 0.25 mg/ml. IC50 for C1 = 0.015 and C2 = 0.1 as shown in Figure 2d and Table 1. C1 has promising antiproliferative activity against HepG2 cells with 0.015 IC50 values, this value is promising when compared to the 0.07 IC50 value of the same complex on the L6 normal cells, and therefore, C1 indicated more anticancer activity at nontoxic concentrations.

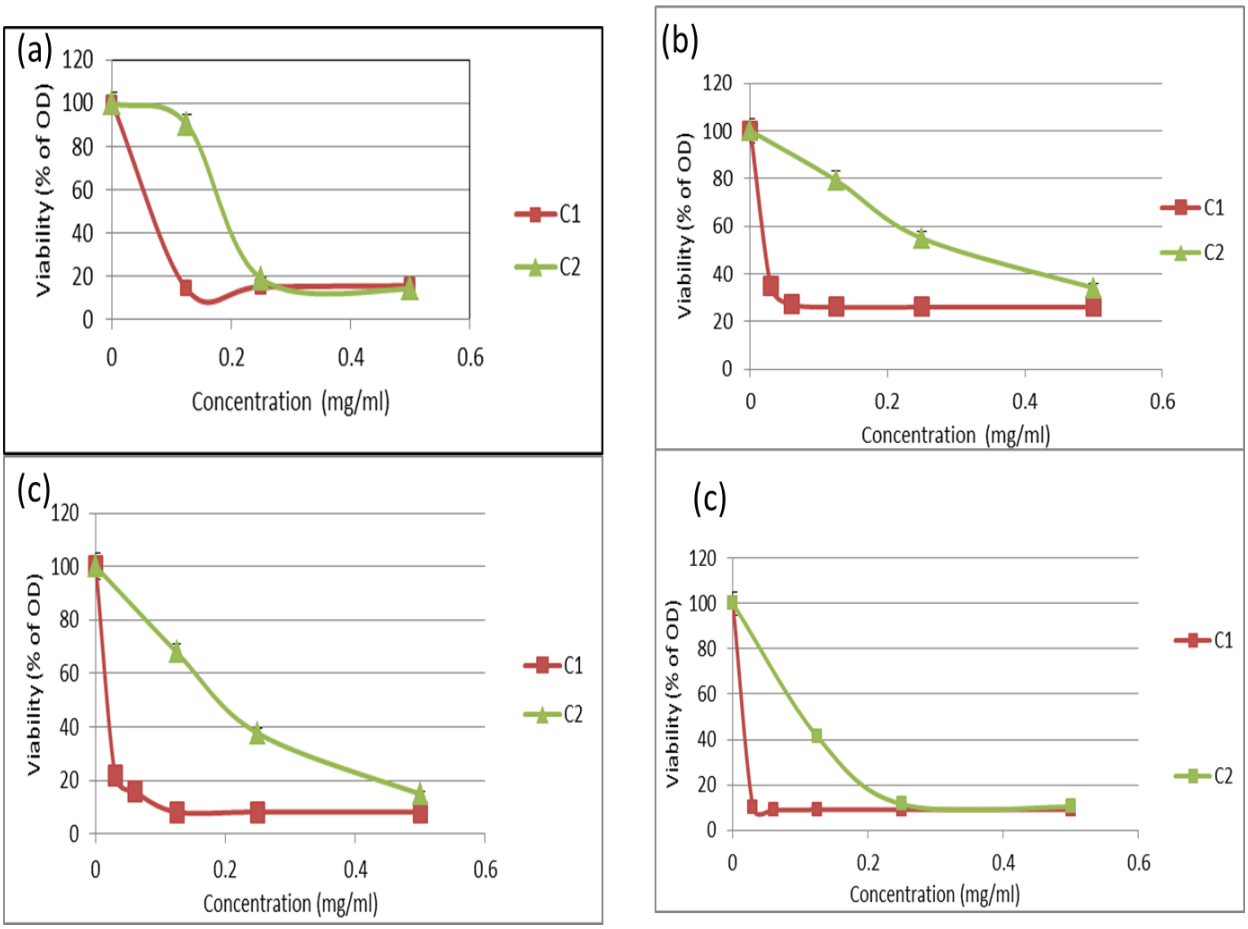


Fig.2: Cytotoxic on (a) L6, (b) HCT, (c) PC3 and (d) HepG2 cells using C1 and C2 complexes

Table 1: IC50 results of C1 and C2 using several cell lines.

Cell line	**IC50 (mg/ml)**	
	C1	**C2**
L6	0.07±0.01	0.18±0.03
HCT	0.03±0.01	0.3±0.01
PC3	0.02±0.01	0.17±0.02
HepG2	0.015±0.003	0.1±0.02

3.3 C1 and C2 complexes cytostatic

Results of *L6 cells* showed that C1 motivated cytostatic effect at very depressed concentration 0.03125 mg/ml and C2 at higher than 0.25 mg/ml. IC50 of the complexes were C1 = 0.02 and C2 = 0.25, as shown in Figure 3a and Table 2. *HCT cells,* C1 induced cytostatic effect at 0.125 mg/ml and C2 induced cytotoxicity at concentrations more than 0.5 mg/ml. IC50 of the complexes were C1 = 0.04 and C2 = 0.37, as shown in Figure 3b and Table 2. By comparing the IC50 values of the two complexes on HCT cells with the IC50 of the same complexes on L6 it indicated that their cytostatic activity on HCT cells considered toxic to the normal cells and not promising. *PC3 cells*, C1 induced cytostatic effect at 0.125 mg/ml and C2 induced cytostatic effect at concentrations more than 0.5 mg/ml. IC50 of C1 = 0.015 and C2 = 0.37, as shown in Figure 3c and Table 2. Actually, C1 exhibited promising antiproliferative activity against the tested cell line with IC50 values of 0.015 this value is promising when compared to 0.02 IC50 value of C2 on L6 indicating that this complex has an anticancer effect at nontoxic concentrations. *HepG2 cells*, C1 induced cytostatic effect at very low concentration 0.03125 and C2 at 0.25 mg/ml. IC50 of C1 = 0.015 and C2 = 0.15, as shown in Figure 3d and Table 2. C1 have promising antiproliferative activity on HepG2 cells with IC50 values of 0.015. This value is promising when compared to 0.02 IC50 value of C2 on L6 reflecting such complex with higher anticancer activity at nontoxic concentrations.

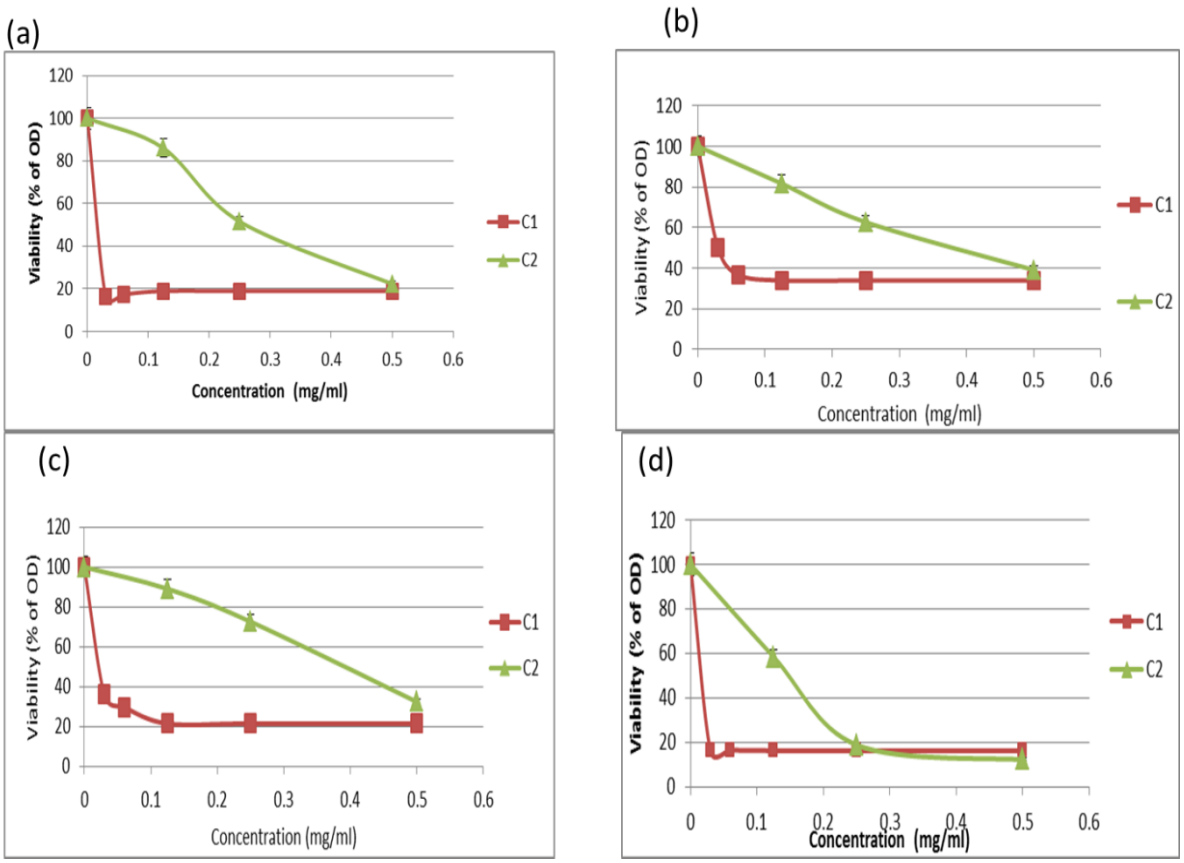


Fig. 3: Cytostatic experiment on (a) L6 (b) Hct, (c) PC3 and (d) HepG2 cells using C1 and C2 complexes in a humidified atmosphere, cells incubated with the complexes for 24 hours.

Table 3. Cytostatic result of C1 and C2 complexes on the studied cell line.

Cell line	IC50 (mg/ml)	
	C1	C2
L6	0.02±0.001	0.25±0.02
Hct	0.04±0.003	0.37±0.04
PC3	0.015±0.002	0.37±0.04
HepG2	0.015±0.002	0.15±0.002

3.4 MTT color sensation

The activity of the complexes against the cancer cells was monitored primary following the changes in the colors before and after mix material, as seen in Figure4**,** even by naked eyes one can detect the MTT activities of such complexes

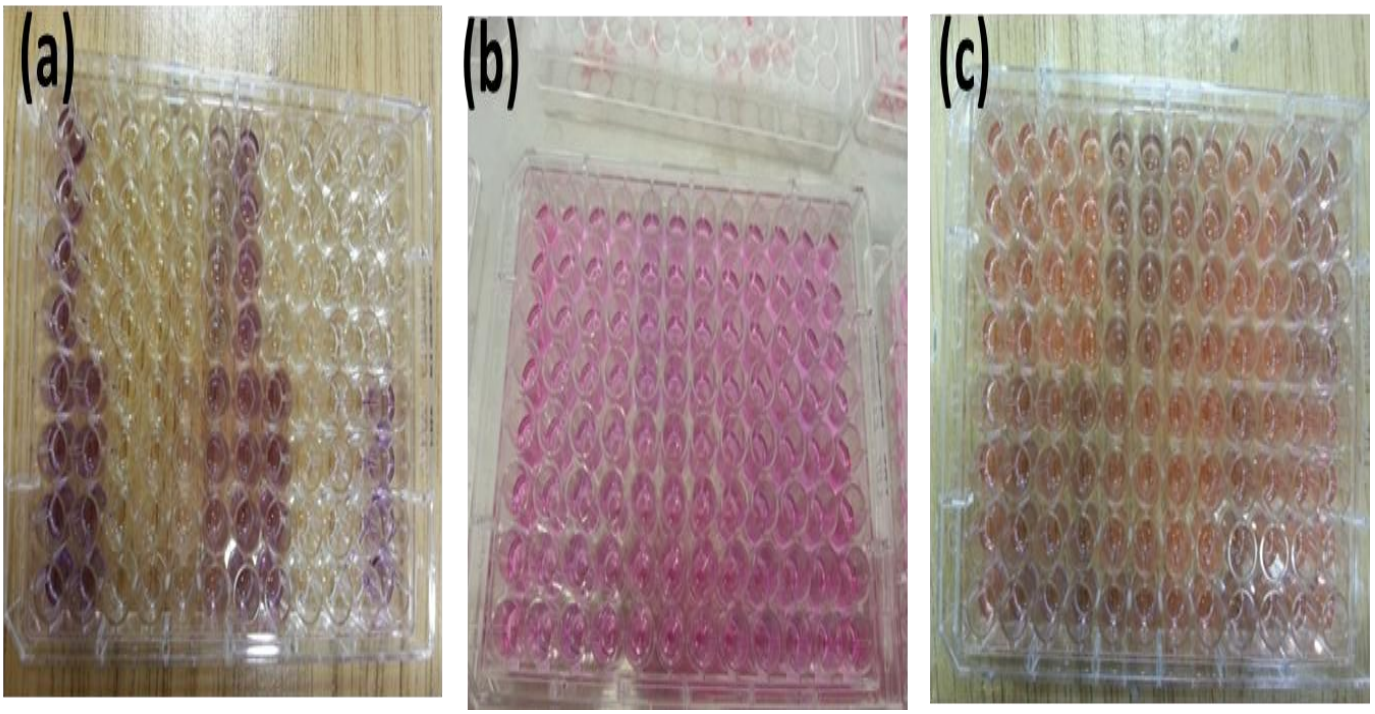


Fig.4. (c) 96 well plates after treatment with C1 and C2 complexes, (b) the same plate after 4 hours incubation with MTT and (c) after 15 minute incubation in dark with isopropanol.

3.4 Microscopic study

The activities of the desired Cu(II) complexes were also supported by performing classical 400X microscopic pictures, for pure HepG2 cells before treatment with Cu(II) complexes and directly after addition of 0.125 mg/ml of C1 or C2 complexes, as seen in Figure 5. Figure 5 showed directly the reducing of HepG2 cells numbers by mixing it with the complexes, which reflected primary these complexes as anti-cancer material.

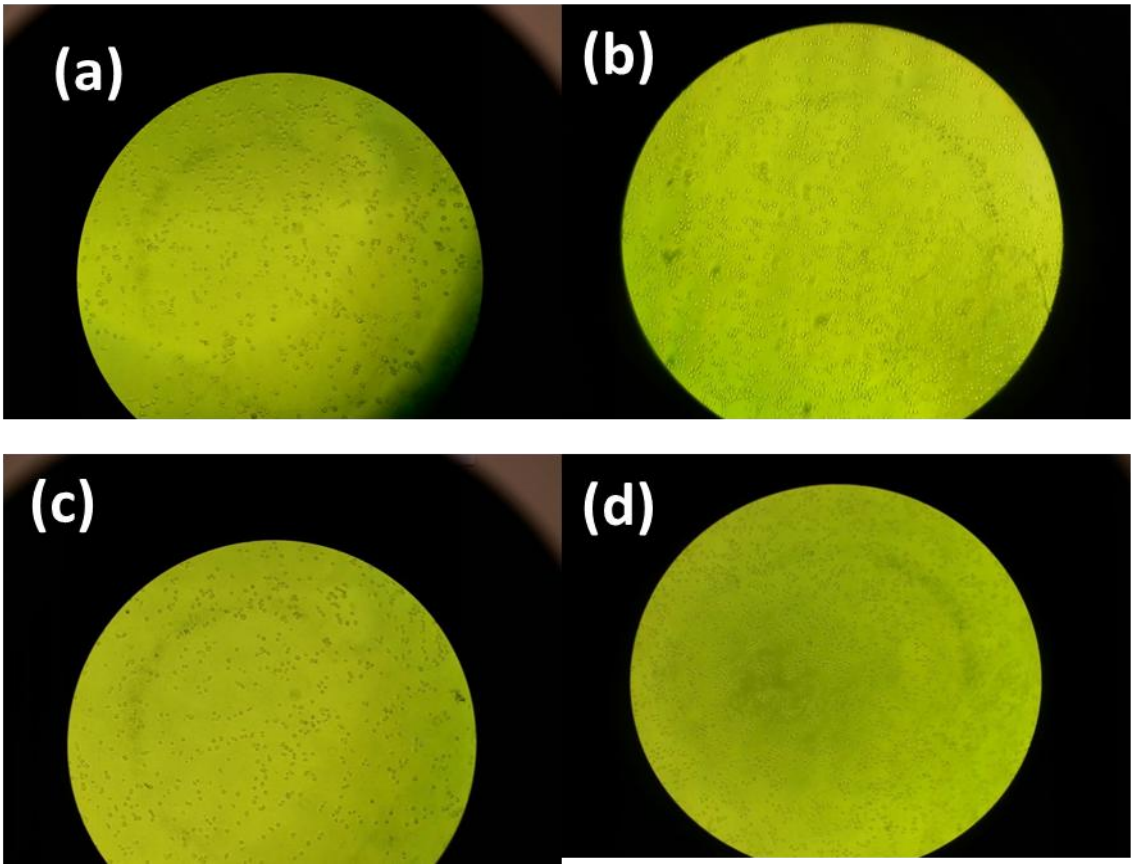


Fig.5. Microscopic pic taken at 400X for: (a) HepG2 cells before treatment, (b) after treatment with 0.125 mg/ml of C1, (c) HepG2 cells before treatment and (d) HepG2 cells after treatment with 0.125 mg/ml of C2.

CONCLUSION

The starting material of [Cu(dipn)Br]Br complex didn't induce cytotoxicity or cytostatic effect which means that the combination of diamine ligands to the [Cu(dipn)Br]Br in order to prepare C1 and C2 complexes played the critical role in enhancing the cytotoxicity and cytostatic effectiveness against L6, PC3, HCT and HepG2 cancer cell lines. C1 and C2 reflected promising anti-proliferative agents results in nontoxic levels used the desired cancer cell lines. In general, C1 complex induced higher cytotoxic and cytostatic effects at lower concentrations compared to C2, which can be resonated to its five-membered ring

structure shape-selectivity priority, therefore, we strongly recommend to use several similar complexes in order to prove this proposition.

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