

Study of the cytotoxic effect of new transition metals complexes: Cobalt (II) and Cadmium (II) on cancer cell line (Hep2) comparison with two anticancer drugs

¹Jinan Hussein Murtadha, ²Iman Hashim Abdul Razzaq

³Mahasin Faisal Alias

^{1,2,3}Department of chemistry, College of Science for Women, University of Baghdad.

Abstract: The present study involved the cytotoxic effect of cobalt complex with formula $[CoL(H_2O)NO_3] \cdot 4EtOH$ where $L =$ Nitro [5-(P-nitro phenyl)-4-phenyl-1,2,4-triazole -3- dithiocarbamate hydrazide] aqua cobalt (II). Ethanol (4) and cadmium complex with formula $[CdL_2] \cdot 1/2 H_2O$ where $L =$ Bis [5-(P-nitro phenyl) – 4-phenyl -1,2,4- triazole -3- dithiocarbamate hydrazide] cadmium (II) hydrate (0.5) on the growth of hepatocellular carcinoma (Hep2) cell line by using in vitro system comparison with two anticancer drugs (Cyclophosphamide and Cisplatin) as appositve control. The cancer cell line were treated with different concentrations for each two complexes depending on concentrations of positive control after 48 hour exposur time. Our data reveals the highest of inhibition rate was reached to 38.49% and 34.65% for each cobalt (II) and cadmium (II) complexes at $1000 \mu g/200 \mu L$ comparison with cyclophosphamide. There were no significant differences between two complexes at two concentrations (500 and 1000) $\mu g/ 200 \mu L$. The results indicate the highest rate of inhibition level was 35.73% of cadmium complex and 29.65% of cobalt complex at $125 \mu g/200 \mu L$ comparison with anticancer drug Cis-platin. The mean value of decreasing percentage of cobalt (II) complex was reached to (- 7)% and cadmium (II) complex was reached to (- 15)% at the same concentration.

Keywords: Cis-platin; Cyclophosphamide; Cobalt(II)complex; Cadmium(II) complex.

I. Introduction

Transition metals have an important place within medicinal biochemistry, research have shown significant progress in utilization of transition metal complexes as drugs to treat several human disease like carcinoma, lymphomas, infection control, anti- inflammatory, diabetes and neurological disorders [1]. Nickel, cadmium, cobalt and arsenic compounds are well – known carcinogens to humans and are well-known carcinogens to humans are experimental animals , even through their DNA damaging potential are rather weak, they interfere with the nucleotide and base excisions repair at low, non cytotoxic concentrations [2]. Some heavy metals cations e.g. Hg^{+2} Cd^{+2} and Ag^{+1} from strong toxic complexes which make them dangerous for any physiology function [3]. Cadmium has been classified as a carcinogen by International Agency for Research in Cancer (IARC), the half life of cadmium in the human body is more than 20 years. So it has enough time to accumulate and cause tissue damage in several organs including lungs, liver, kidney, cardiovascular and nervous system due to occupational intoxication[4]. Some investigation showed that the development of new agents with modes of action different from Cis-platin is possible, thus complexes with iron, cobalt or gold central atom[5]. Cobalt- alkyne complexes represent a new class of antiproliferative drugs with high activity on cell lines derived from human solid tumors[6]. Several alkyne cobalt carbonyl complexes inhibited the growth of human melanoma and lung carcinoma cell [7]. Certain metals are also essential for human health, cobalt plays a critical role in the synthesis of vitamin B12 [8]. Transition metal complexes with dithiocarbamates as ligands have been extensively investigated and were interested in many fields, as flotation agents and antifungal agents[9]. Cis – platin has been used as a first – line therapy for several cancers, including testicular, ovarian, cervical, head and either alone or in combination with other anticancer agents [10]. Cyclophosphamide known as cyclophosphane is a nitrogen mustard alkylating agent from the oxaphosphorine group [11].

Cyclophosphamide is used to treat cancers and autoimmune disease. It is used to quickly control the disease and it was sever and life- threatening adverse effects, including acute myeloid leukemia, bladder cancer hemorrhagic cystitis and permanent infertility, especially at higher doses [12].

II. Materials and Methods

1. Cyclophosphamide (CP) anticancer drug.
The anti- cancer drug was provided by Baxter (Germany) (200mg/10ml).
2. Cis – platin (Cis –Pt) drug
The anti- cancer drug was provided by Ebew (Austria)

3. New transition metals complexes, cobalt (II) and cadmium (II) were provided by Hashim [13]. These complexes were dissolved 200mg in 10ml of normal saline (the same concentration of cyclophosphamide). We prepared three concentrations (2000, 1000, 500) $\mu\text{g} / 200 \mu\text{l}$ for both cyclophosphamide and new complexes. The same complexes were dissolved 10mg in 90 ml of normal saline depended on concentration of Cis-pt and we prepared the three concentrations (250, 125, 62.5) $\mu\text{g}/200 \mu\text{L}$ for both Cis- pt and new complexes.

4. Study of cytotoxic effect on cancer cell line.

The method was used to investigated the effected of new transition metals complexes on Hepatocellular carcinoma (Hep2). The cancer cell line was provided by center of biotechnology research center of Al-Naharin university. All solutions are prepared at the same center and culturing tissues were studied in vitro under optimum conditions by the same center. The growth media used in tissue culture technique was MEM (Minimum Essential Media) was provided by Fetal calf serum (10%) to form a confluent monolyer, then subculture to discard the previous growth medium and the cells washed with sterilized phosphate buffer solution(PBS) by autoclave at 121°C for 15 min and addition 2-3 ml of trypsin – versene solution was added for 3-5 min and moving the culture flask kindness. The trypsin – versene solution to discard and cells incubated at 37 °C until the cell separation from ground flask, added new growth media and redistribution of cells at the microtiter and incubated at 37 °C [14].

III. Cytotoxicity Assay

In this assay, the cancer cell line (Hepatocellular carcinoma) (Hep2) was treated with new transitions metals complexes, cobalt (II) and cadmium (II) by using three concentrations (62.5, 125,250) $\mu\text{g} /200 \mu\text{L}$. Immediately added 25 ml of trypsin – versene solutions in to culture bottle and 20 ml of culture medium which contains 10% of serum to provide the suspended cells, mixed very well and 0.2 ml was added to each microtiter. The plates were incubated at 37 °C for 24 hour to form monolayer, then the previous culture medium which presents into the plates was discarded. The volume 0.2 ml of the solutions under study were added and these three preparations repeated as negative control (Hep2 with buffer solution) and incubated at 37 °C for 48 hour exposure time.

The culture medium was discarded from microtiter plates, 0.2 ml of crystal violet solution was added to wells and the plates were incubated for 20 min at 37 °C. The plates were washed gently with distilled water and left to dry. At the end of assay the plates were examined by ELISA reader at 492 nm (transmitting wave length). Only viable cells able to take stain while the dead cells were not. The inhibition percentage was measured according to Gao et al[15] as follow:

$$\text{Inhibition percentage \%} = \frac{\text{Absorbance of negative control} - \text{Absorbance of test}}{\text{Absorbance of negative control}} \times 10$$

Statistical analysis

The data were analyzed using analysis of variance ANOVA. Student T-test was used to assess significant difference among means at level ($P < 0.05$)[16].

IV. Results and Discussion

Study of cytotoxic effect on growth cell line Hep2

The cytotoxic effect of two transition metals complexes were studied on cancer cell line Hep2 and compared with two anticancer drugs cyclophosphamide (CP) Cis-platin (Cis-pt)

- Cyclophosphamide (CP)

*** Inhibition rates**

The results showed significant differences between three concentrations ($P < 0.05$) when cancer cell line treated with cobalt (II) and cadmium(II) complexes comparison with positive control CP after 48 hour exposure time. The highest of inhibition rate reached to 38,9% after treatment with cobalt complex at 1000 $\mu\text{g} / 200 \mu\text{L}$ and the lowest of inhibition rate at concentration 500 $\mu\text{g}/ 200\mu\text{L}$ was reached to 15.67% as shown in table(1).

Table (1): Comparison of the inhibition percentage between the two new complexes and cyclophosphamide (CP) on Hep2 cancer cell line.

Treatment Conc. $\mu\text{g}/200\mu\text{L}$	Inhibition rates % (mean \pm standard deviation SD)		
	CP	Co-complex	Cd-Complex
500	A,b 14.32 \pm 2.25	A,c 15.67 \pm 2.91	A,b 20.42 \pm 3.30
1000	A,a 31.42 \pm 2.75	A,a 38.49 \pm 0.96	A,a 34.65 \pm 3.29
2000	AB, a 25.10 \pm 2.48	B,b 23.60 \pm 4.52	A,a 32.92 \pm 3.22

--Differences letters (A,B,C) significant differences ($P < 0.05$) as comparable between columns.

--Differences letter (a,b,c) significant differences ($P < 0.05$) as comparable between rows.

The results indicate no significant differences Co- complex and Cd- complex in two Concentrations (5000, 1000) $\mu\text{g}/200 \mu\text{L}$ comparison with cyclophosphamide.

*** The increasing and decreasing of inhibition percentage.**

The fig (1) was shown the percentage of increasing and decreasing of inhibition rate on cancer cells were treated with cobalt (II) complex and cadmium (II) complex comparable with positive control (CP). The increased percentage was 22.50% of cobalt complex while the cadmium complex reached to 10% at 1000 $\mu\text{g}/200\mu\text{L}$. The highest percentage of increasing was reached to 42.50% when the cancer cell line treated with cadmium complex at lower concentration 500 $\mu\text{g} / 200\mu\text{L}$ comparison with 9% of cobalt complex in the same concentration. The percentage of decreasing inhibition was (-6%) at 2000 $\mu\text{g}/200\mu\text{L}$ when the cancer cell line treated with cobalt complex.

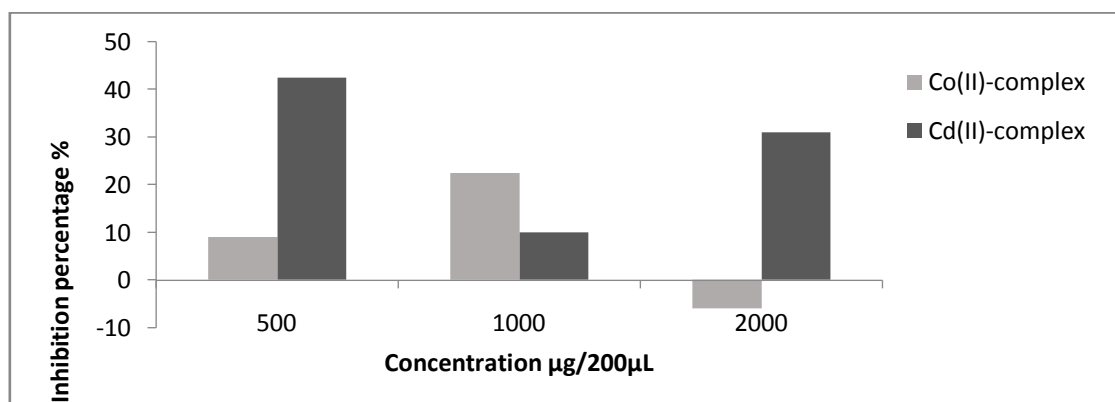


Fig. (1): Increasing and decreasing the percentage of complex $\mu\text{g}/200\mu\text{L}$ inhibition for two transition metals comparison with positive control (CP)

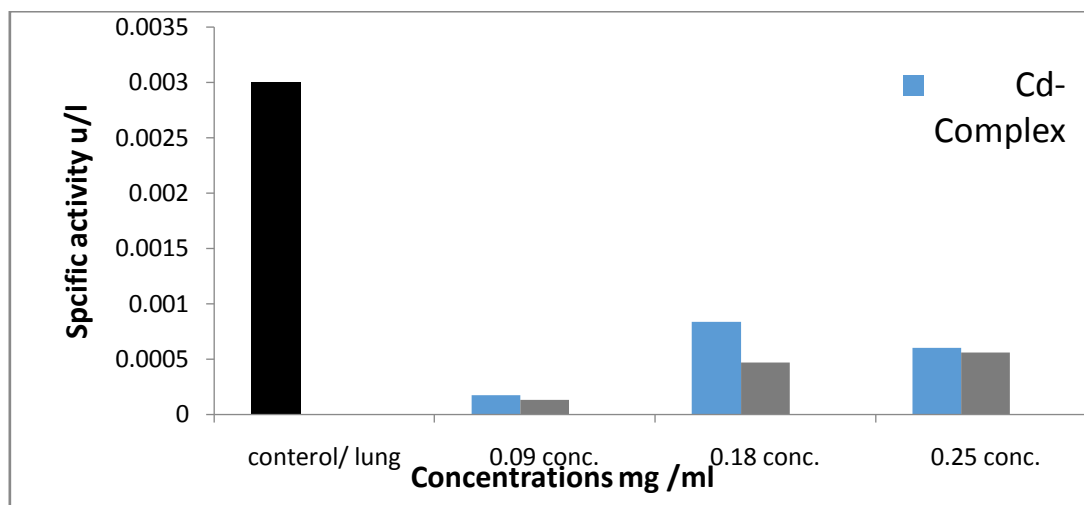
On previous study, we indicated the cytotoxic effect of the same cobalt (II) complex on albino / female mice *in vivo* system in different organs and the result showed the highest of inhibition rate of GPT specific activity in Liver at 180 $\mu\text{g}/\text{mouse}$ comparison with the same anticancer drug (CP) as shown in table (2) [17]. Then, the same effect were noticed in lung at same concentration when the mice were treated with cadmium (II) complex as shown in fig(2) [18].

Table (2): Effect of cobalt (II) complex and cyclophosphamide on GPT (Glutamate Pyruvate Transaminase) specific activity of liver female mice comparison with normal control .

$\mu\text{g}/\text{mouse}$	GPT specific activity by U /mg protein $\times 10^{-2}$. (Mean \pm standard deviation)			
	90	180	250	Inhibition rate of cobalt (II) complex comparison with normal control
Control	A,a 27.2 \pm 0.002	A,a 25.4 \pm 0.0022	A,a 26.8 \pm 0.0022	%95.84
CP	B,a 3.0 \pm 0.00351	B,a 2.57 \pm 0.0070	B,a 2.17 \pm 0.00201	%82.41
Cobalt (II) complex	B,a 1.13 \pm 0.0032	B,b 4.467 \pm 0.007	B,c 2.170 \pm 0.00304	%91.90

Differences A, B, C are significant (P < 0.05) to comparison columns

Differences a,b,c are significant (P < 0.05) to comparison row



Fig(2):The specific activity of GPT enzyme of lung of female mice treated with different doses of cadmium (II) complex compared to the CP and control.

- Cis-pt anti cancer drug

***Inhibition rates**

The treatment of cancer cell line (Hep2) as shown in table (3), there were significant differences (P < 0.05) between two concentration (62.5 and 125) µg/200 µl of cobalt (II) and Cis- pt drug .The highest inhibition rate reached to 42.23% of Cis-pt, 35.73% of cadmium complex and 29.65% of cobalt at 125 µg/200µl. the statistical results in the same table illustrated there were no significant differences between two new complexes comparison with Cis-pt at high concentration 250µg/200 µl. The results indicate the lowest inhibition rate was reached to 13.66% when the cancer cell line was treated with cobalt (II) complex at 62.5µg/200µl comparison with Cis-pt.

Table(3):The correlation between inhibition rate and new transition metals complexes comparison with Cis-pt.

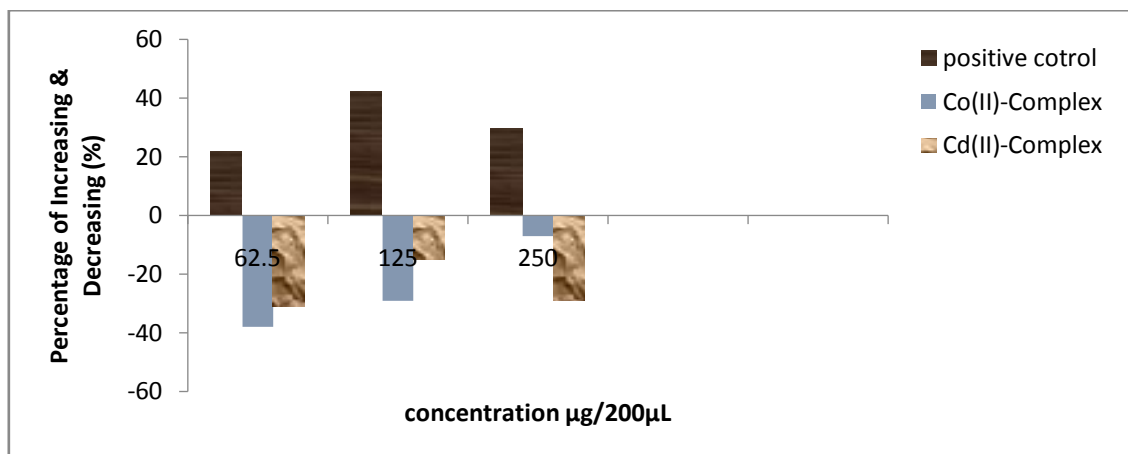
Treatment Conc. µg/200µL	Inhibition rates% (mean± standard deviation SD)			
	Cis-pt	Co-complex	Cd-complex	P
62.5	A,b 22.06±3.12	B,a 13.66±1.92	AB,b 15.13±2.29	P < 0.05
125	A,a 42.23± 2.71	B,a 29.65±2.31	AB,a 35.73±3.58	P < 0.05
250	A,a 29.59± 1.76	A,a 27.48±4.69	A,b 22.48±1.34	P < 0.05

Differences A,B,C are significant (P < 0.05) to comparison columns

Differences a,b,c are significant (P < 0.05) to comparison rows

***The increasing and decreasing of inhibition percentage.**

The mean value of two new complexes cadmium (II) and cobalt (II) comparison with anticancer drug Cis-pt presented in fig (3). The result indicate an evidence decreasing inhibition when cancer cell line were treated with cobalt(II) complex was (-7%) at250µg/200µL, while this decreasing was doubled to (-15%) at 125 µg/200µL when cancer cell line was treated with cadmium (II) complex.



Fig(3):Inhibition percentage of increasing and decreasing of Co(II) and Cd(II) complexes comparison with Cis-pt.

The results clearly indicate that cobalt (II) and cadmium (II) complexes were higher cytotoxic on cancer cell line (Hep2) at 1000 µg/200 µl comparison with cyclophosphamide, that could be attributed that cobalt ion could be induced the damage of proteins macrophage – H like cells *in vitro* [19]. The effect of dithiocarbamate ligand was anti neoplastic was attributed to pro-apoptotic relate mitochondrial membrane permeabilization, dithiocarbamates were reported to induce apoptosis in thymocytes by raising the intracellular level of redox-active copper and glutathione [20]. On the other hand, our previous study investigated the effect of cobalt complex *in vivo* system as shown in table (2), the enzymatic activity changes in the liver of albino/mice that reflect the damage degree of animal liver, the accumulation of toxic substances in animal body was cause grievous injury in hepatic tissue and then would cause animal hepatase activity changes [17]. The cadmium (II) complex presented the same cytotoxic effect on cancer cell line (Hep2) in the same concentration, the cadmium affect cell proliferation and differentiation, cell cycle progression, DNA synthesis and repair, apoptosis and other cellular activities [21]. Michael *et al*, studied the inhibition of DNA repair Process by cadmium enhances the genotoxicity of other agents and may be contributed to the tumor initiation by this metal [22]. The cytotoxicity effect of cyclophosphamide was similar to cobalt (II) and cadmium (II) complexes, we suggest the anticancer drug (CP) have inhibition effect due the cross link DNA by adding alkyl group to the guanine base of DNA at N=7 position of the imidazol ring that induce inhibition of DNA replication leading to cell death [23]. Our results were agreed with our previous study about cytotoxic effect of cadmium complex on GPT specific activity in lung of albino/mice more than of other organs [18]. The cytotoxic effect of new metals complexes was similar to Cis-pt that was believed due to its interaction with chromosomal DNA and the aquated form of Cis-platin is potent electrophil and reacts with a nucleic acids and sulphhydryl groups of proteins [2].

V. Conclusion

The study showed the new metals complexes; cadmium (II) and cobalt (II) have cytotoxic effects on Hep2 cell line for three concentrations after 48 hour exposure time by the highest inhibition rates at 1000 µg/200 µl and 125 µg/200 µl comparison with cyclophosphamide and Cis-pt respectively.

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