Study of the Toxic effect of new Cadmium (II) complex [CdL2]. 1/2H_2O on GPT and ALP activity in some organs of female mice comparable with anitumor drug Cyclophosphamide (CP)

Iman H. Abdul Razzaq*

Received 13, January, 2014
Accepted 7, April, 2014

Abstract:

Cadmium has been known to be harmful to human healthy , manily Via contaminated drinking water , food supplies , tobacco and industrial pollutant . The aim of this study was to determine the toxicity of new Cadmium (II) complex **(** Bis[5- (P- nitrophenyl) – ˉ4 – Phenyl- 1,2,4- triazole -3- dithiocarbamatohydrazide] cadmium (II) Hydra (0.5) and compare it with anticancer drug cyclophosphamide (CP) in female albino mice . This complex causes to several alterations in Enzymatic activity of Glutamate Pyruvate Transaminase (GPT) and Alkaline Phosphatase (ALP) in three organs after the treatment of mice with different doses of a new cadmium (II) complex (0.09 / 0.25ml , 0.18/ 0.5ml and 0.25mg /0.7 ml /30 gm of mouse) for three days by the decreased of Glutamate Pyruvate Transaminase activity in lung , Liver and kidney , while the Alkaline Phosphatase activity was increased in kidney , lung and liver . The results were indicated that no significant differences ($P < 0.05$) in Glutamate Pyruvate Transaminase activity between the treatment of mice with cadmium (II) complex and cyclophosphamide (CP) in the liver , kidney and Alkaline Phosphatase activity in the liver at each three doses .

Key words: Cytotoxicity , Cadmium(II) Complexes ,Cis-platin

Introduction:

Chemotherapy is the use of synthetic chemicals or drugs extracted from plants these compounds are toxic against cancer cells , inhibiting their reproduction and division [1] . Cyclophosphamide is among most utilized drugs in chemotherapy drug usually given to treat Lymphomas leukemias , lung cancer and breast cancer , it may also be used to treat many other types of cancer [2] . Some heavy – metal cations e.g. Hg^{+2}, Cd^{+2} and Ag^{+} from strong toxic complexes which make them too dangerous for any physiology function [3] . For a long time cadmium has been considered a non – genotoxic carcinogen , as it is only weakly matagenic in bacterial and mammalian cell test systems . Filipic , *et al*. [4] presented evidence that when assayed in a test system , in which both intragenic and mutations could be detected , cadmium acts as a strong mutagen which induces predominantly multilocus detections . Elemental cadmium and cadmium compounds have been known to be harmful to human health [5,6]. Pierpaoloetal [7] were investigated the characterize of cadmium – induced apoptotic response in normal and tumor cells derived from human prostate epithelium at dose relevant to human exposure, $CdCl_2$ to induce cytotoxic in the human lung carcinoma cell line in the lower micromolar concentrations

*College of Scince for women , Chemistry Department , University of Baghdad

[8] . Transition metal complexes with dithiocarbamates as a ligands have been extensively investigated and were of interest in many fields ;as flotation agents and as antifungal agents [9]. Enzymes are biochemical macromolecules that catalyst accelerate metabolic processes of organoisms , thus a slight variation in enzyme activity would affect the organism [10].Poirier *et al.* ,[11] , studied the effect cadmium(II) in marine bacterium *Pseudomonas* on ALP and were observed the metal ion inhibition of ALP. In the present experiments the toxic influence of a new complex (Bis [5- (P-nitrophenyl)-4- phenyl-1 ,2,4- traizole -3-dithiocarbamato hydrazide] cadmium (II) hydra (0.5)] on the activity levels of (GPT and ALP in the liver , kidney and lung tissues of female albino mice were assessed on three doses after three days of the treatment comparable with anticancer drug cyclophosphamide.

Methods and Materials :

- Albino female 8-12 weak old mice (weighting 30 ∓ 5 gm) were kept at 21 ∓ 2 C°, fed with pellet mice diet and exposed to 12 h light /12h dark cycle . Group 1 was used as a control . The mice of the experimental groups were divided in two groups : Group2 : treated with different doses of cyclophosphamide (0.09 / 0.25 ml, 0.18 /0.5 and 0.25 mg /0.7 ml) /30gm of mouse) and group 3 : to cadmium complex and the single dose was injected in intraperitoneally .
- Cyclophosphamide (CP) (200 mg /10 ml) the anticancer drug was provided by Baxter (Germany) and were prepared from this solution stock solution (2.7mg/7.5ml of normal saline).The three concentrations were prepared from this solution (0.09 , 0.18 and 0.25) mg and stored at (2-8) C° until used in tests
- New complex cadmium (II) drug was provide by Hashim [1]. (200 mg of this complex was dissolved in 10 ml of normal saline) and were prepared the same stock solution and doses used for cyclophosphamide drug.

After three days , all the mice of each group were sacrificed and liver , kidney and lung tissues were removed . The tissues were then weighted and stored an Eppendrof tubes containing normal saline (NS) at -70 C° until processing .

liver , kidney and lung tissues were prepared according to the method AL-Shami[12] , then 80% was extracted from the total activity of enzyme . The tissues were homogenized using a glass homogenizer with equal quantity of dry sand (wash by diluted aceteic acid 5% and washed with distilled water) and mixed very well until homogeneous solution , then added the buffer solution (pH=7.4) 2ml for each 1ml of tissue (weight) and mixed well until homogeneous solution. After that added Butanol tissue (1:1) ml with mixing for 15 min . The tubes were incubated in a water bath at 37 C° and were centrifuged at 3700 rpm , for 10 minutes . The supernatant was then used for quantifying the enzymes .

- **Enzyme Assays**

The supernatant was measured using colorimetric determination by using GPT kit and ALP kit provided Randox Company and the enzymes specific activity were expressed (u / mg).

- Glatamate pyruvate transaminase (GPT) Kit

L- Alanine 2- oxoglutarateamino-transferase was measured with 2,4 dinitrophenylhy-drazine according to method of Razzaq [13]

- Alkaline phosphatase (ALP) Kit

was assessed using 4- aminoantipyrine according to method of R ikabi and Jawad [14].

-Protein estimation

Protein concentration in the body tissue was estimated using Biuret method [15]. Protein residue was obtained from supernatant produced after centrifugation at 3700 rpm from 10 minutes . Using 0.5gm /100 ml Humain serum albumine (Biotest – Germany) as a standard .

- Statistical Analysis

The data were expressed as the mean ∓SD and were analyzed by mean of two way analysis of variance (ANOVA-Test) .Statistical evalution of data was done following variance test (ANOVA) .A difference was considered significant at $P < 0.05$ [16].

Results and Discussion:

- Glutamate pyruvate Transaminase (GPT) activity of tissues; **Liver, kidney and lung**.

The mean value of specific activity of tissues from mice after the treatment with cadmium (II) complex and their cyclophosphamide comparably with controls and presented in Figure (1,2 and 3) . The results present an evidence that treatment with cadmium (II) complex showed the inhibition in enzymatic activity at three doses was reached to 94.23 % in the lung , 93.95% in the liver and 90.96% in the kidney comparably with control at 0.09 mg / mouse. The results also the mean values of GPT specific activity after the treatment with a new complex which was found to be not significant differences ($P < 0.05$) at each three doses in the liver , kidney and lung . these effects were similar to the effect of anticancer drug .

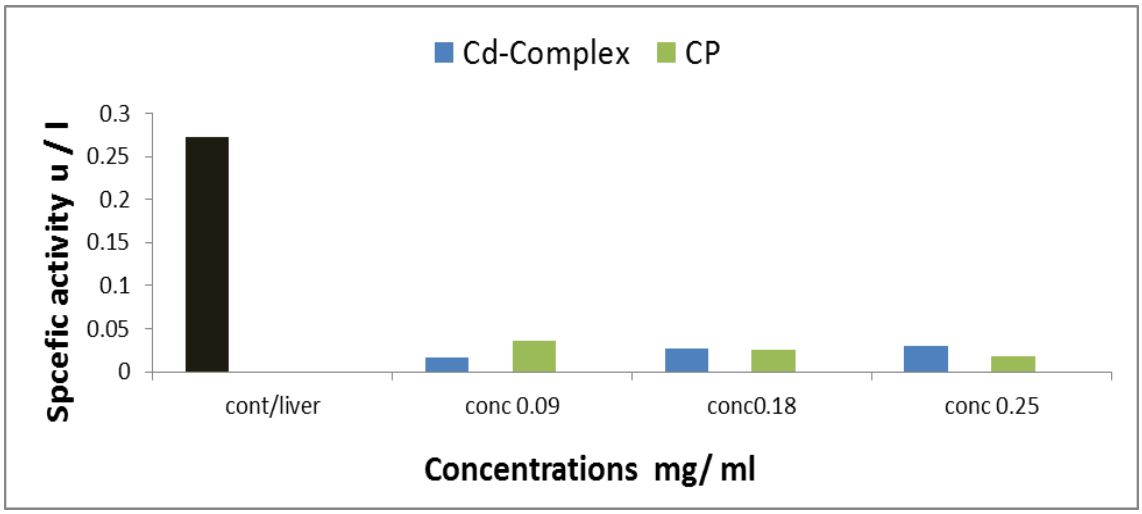

Fig.(1): The specific activity of GPT enzyme of liver of female mice treated with different doses of cadmium (II) complex compared to the CP and control.

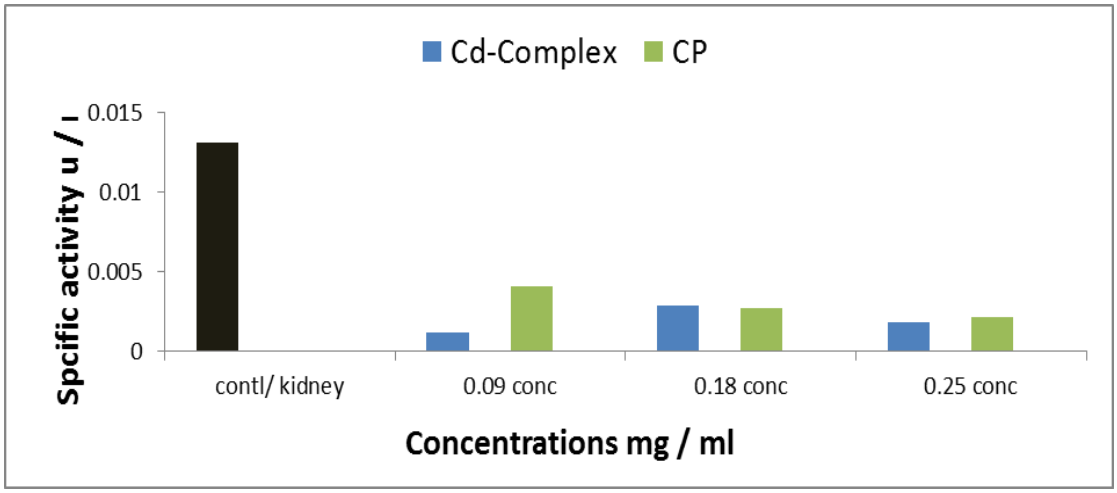

Fig. (2): The specific activity of GPT enzyme of kidney of female mice treated with different doses of cadmium (II) complex compared to the CP and control.

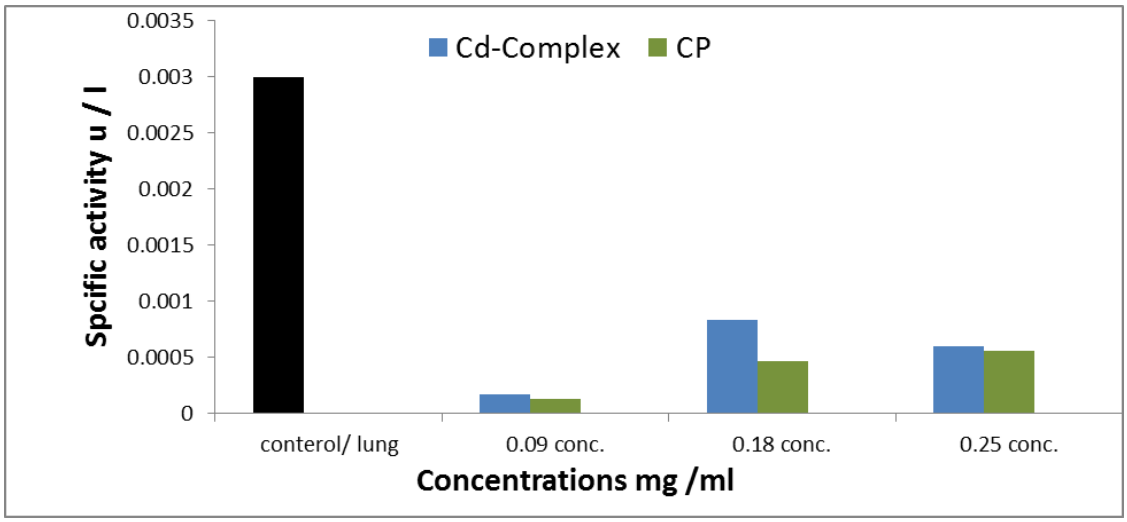

Fig. (3): The specific activity of GPT enzyme of lung of female mice treated with different doses of cadmium (II) complex compared to the CP and control.

-Alkaline phosphatase transaminase (ALP) Activity of tissues: liver , kidney and lung

The data of AlP – specific activity of liver , kidney and lung from mice treated with cadmium (II) complex comparable with anticancer drug cyclophosphamide are summarized in Fig (4) and Table 1 and 2) . The results show an evidence that was not significantly ($P < 0.05$) in liver and kidney at 0.18 and 0.2 mg / mouse , while there were highly significant differences at lower concentration 0.09 mg / mouse . The liver (ALP) activity vules were showed a relative activation of about 36.81 % ,245.83% and 139.38 respectively on day three after the mice treatment with cadmium (II) complex at three concentrations comparable with controls , while the activity value were increased in Lung and kidney more than liver . Also the results indicated there was highly significant ($P < 0.0.5$) when the mice were treated with cadmium (II) Complex at 0.09 mg and 0.18 mg / mouse in the liver , kidney and lung , while there were highly significant differences for mice treatment with CP drug at three doses in kidney , lung tissues and there were not significant differences in the liver at the same doses .The effect of cyclophosphamide

was similar to the effect of cadmium complex at each three doses as shown in the Fig .(1) and the tables (1 and 2) : AIP specific activity of liver , kidney and lung of female mice treatment with different doses of cadmium (II) complex compared to the cyclophosphamide (CP) and control.

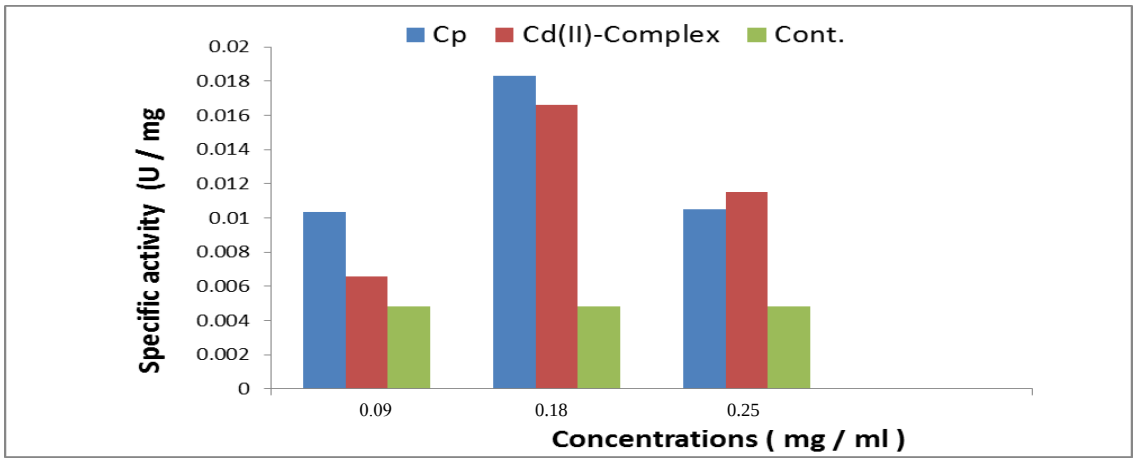

Fig. (4) : The specific activity of ALP enzyme of liver of female mice treated with different doses of cadmium (II) complex compared to the CP and control

Table(1) : The specific activity of ALP enzyme of kidney of female mice treated with different doses of cadmium (II) complex compared to the CP and control.

Dose / Treatment	Alkaline phosphatase (AIP) specific activity(unit/ mg protein) Mean ± SD		
	0.09mg/mouse	0.18mg/mouse	0.25mg/mouse
Control	A,a 0.00138 ± 0.00020	A,a 0.00138 ± 0.00020	A,a 0.00138 ± 0.0002
Cadmium(II) complex	B,a 0.11500 ± 0.02291	B,b 0.2280 ± 0.03759	B,a 0.14467 ± 0.00666
Cycloophosphamid (CP)	C,a 0.01263 ± 0.00327	B,b 0.27767 ± 0.02011	B,C 0.12700 ± 0.02227

Differences A,B,C are significant ($p < 0.05$) to compression colums
Differences a,b,c are significant ($p < 0.05$) to compression rows .

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

Dose / Treatment	Alkaline phosphatase (AIP) specific activity(unit/ mg protein) Standard error ± average		
	0.09mg/mouse	0.18mg/mouse	0.25mg/mouse
Control	A,a 0.000383 ± 0.00030	A,a 0.000383 ± 0.000030	A,a 0.000383± 000030
Cadmium(II) complex	B,a 0.008633 ± 0.000764	B,b 0.018133 ± 0.001850	B,a 0.01670 ± 0.004034
Cyclophosphamid (CP)	C,a 0.00770 ± 0.002307	B,b 0.002933 ± 0.000513	B,C 0.011567 ± 0.003044

Differences A,B,C are significant ($p < 0.05$) to compression colums.
Differences a,b,c are significant ($p < 0.05$) to compression rows .

No mortality was observed during the three days treatment period in the mice treated to three concentrations of cadmium complex (II). There is a clear association between the mice treatment with cadmium (II) complex and the significant depletion of GPT activity at most doses in different tissues compared to cyclophosphamide could be attributed to the increased the permeability of the cell as well as the direct effect of the heavy metal on tissues[10]. Rikabi and Jawad [14] showed the damging effect of cadmium in the liver manifestested by an increase of AST,ALT and ALP, which specific marker of the liver cell damge . The decreased activities of GOT , GPT ,ACP and AIP indicate disturbance in the structure and integrity of cell organelles , like endoplasmic reticulum and membrane transport system , such damage to cell organelles has been reported in various studies [17] cadmium toxicity occurs through interactions with proteins that subsequently cause dysfunction of protein complex and organelles [18].

ALP was observed to increase on the three day of treatment in all the treatments with cadmium (II) complex could attributed to Alp react both organic and inorganic compounds , AIP is a metallenzyme which contain in the active site Zn^{+2} and Mg^{+2} ions and compounds , thus the ability of AIP of react these compounds could make this system useful as biomarker of sample toxicity [19]. Aanand et al [20] studied the effect of heavy metals , copper , zinc and lead on the enzyme activity of green mussel pernaviridis and observed the activity of ALP was increased with compared to that control animals after seven days of treatment .cadmium has the ability to produce reactive radical ,resulting in DNA damge ,lipid peroxidation ,depletion, of protein ,sulfhydryls and other effects and chelates of amino acids,peptides and protein complexed with toxic metal [21]. Our previous investigation showed that the dithiocarbamate complexes can be used constructed sensors for the guest molecles , beside, these coordination compounds containing sulfur and Nitrogen atoms are good corrosion inhibitors in acidic media [1] . The present results agrred with ognjanovic *et al*., [22] were studied the liver , kidney,Lung , testes and heart are the target organs following cadmium exposure with the severity of their intoxication dependent on the route , dose and duration of the exposure to the metal .

Conclusion:

The present study showed the new cadmium (II) complex has toxic effect by the inhibition of specific activity of GPT and the elevation of ALP activity in the liver , kidney and lung tissues from female albino mice , this effect was similar to the effect of anticancer drug cyclophosphamide at different concentrations after three days of the treatment .

References :

1. Hashim,C.S .2012 . Synthesis and studying new complexes of some transition metals Ions on RD cell line . M.Sc. Thesis , university of Baghdad , Iraq , 10- 80 .
2. Williams , L. and Wilkins. 2007 .Cadmium induces p53- Dependend Apoptosis in human prostate Epithelial cells, 4thed , 297 .

A. Shriver,F,Atkins,P.W,Overtone, T.L., Rouke, J.P., Weller, M.I. and Arrmstone, F. 2006. Inorganic Chemistery. 4thed, Oxford, , 305-306.

3. Filipic , M., Fatur , T.and Vudrag , M. 2006. Molecular mechanisms of Cadmium induced mutagenicity , Hum . Exp .Toxicol 25 (2) : 67-77.

4. Cod t, J. scheidig , F, Grosse – siestrup , C. , Esche , V. etal .2006 . The toxicity of Cd and resulting hazards for human health, J. Occup .Med .Toxicol 1 (22) : 18-20
5. Ishido , M. and kunimoto , M. 2001 . Regulation of cell fate by cadmium and Zinc , J. Healh . Sci . 47 (1) : 9-13 .
6. Pierpaolo , A., Marco C. Anna , R.V .Altomare,,D.B. etal. 2012 . Cadmium Induces p53 – Dependent Apoptosis in human prostate Epithelial , J.pone.7 (3) : 33647-33648.
7. Pan jehpour , M. and Bayesteh , M. 2008 . The cytotoxic effects of Cadmium chloride on the human lung carcinoma (Calu -6) cell line , Res . Pharm . Sci.3(2) : 49 -53 .
8. Xia , Y.I ., Dong, F.C., B.x., Zhao ,B.X. , et al .2008 .Synthesis and structure – activity Relation ships of novel -1- aryl methyl -3- aryL-IH- pyrazole -5- carbohydrazide Hydrazone Derivatives as potential Agents Against A 549 lung cancer cells , Eur ,J. Med . Chem . 43(11) : 2347 – 2353 .
9. Roy, S. S. 2002. Some toxicological aspects of chlorpyrifos to the intertidol fish *Boleopthalmus dussumieri*. Ph.D .Thesis, university of Mumbai , India, 52-71.
10. Poirier,I.Hammann,P.,Kuhn,L and Bertrand , M. 2008 . Responses of the marine bacterium Pseudomonas fluorescens to an exces of heavy metals :Physiological and biochemical aspects, Sci. Env. 406(1-2) : 76-77.
11. Al-Shami, J. H. M. 2000 .Detection of carcinogenic and mutagenic agents in drinking water using enzymatic and analysis . M.Sc. Thesis,university of Baghdad ,Iraq, 50-51.
12. Razzaq ,I. H. A. 2011.Study of cytotoxic effects of alcoholic Nerium oleander L. extract on female albino mice, J.Bag. Sci. 18(2): 361-362.
13. Rikabi , A .A .and Jawad , A .H. 2013. Protective effect of ethanolic Ginger extract a ganist Cadmium toxicity in male rabbits, Bas .J .Vet .Res . 12(1):15-22.
14. Khawaja ,T., Khan ,H. S., Mukhtar ,N. ,Parveen ,A. and Fareed ,G. 2013 . Production performance ,egg quality and biochemical parameters of threeway crossbred chickens with reciprocal F ,crossbred chickens in sub-tropical environmrnt, .Italine J. Anim. Sci .Vol 12(1 :982-983.
15. Al-Mohammed , N. T.Al-Rawi ,K .M.,Younis ,M.A and Al – Morani ,W . K .1986 . Principle of statistics , J . mous.univ.13(2): 55 - 58.
16. Nchumbeni , H; Reza ,D., Kulkarni , B.G and Bhavita , C. 2007 . Effect of Arsenci on the Enzymes of the ROH4 Carp , Labeo Rohita, J. supp. 14 :17 -19 .
17. Isamu , A , Anwar , H.andMacus , T.K. 2008. GSH Level Increases Cadmium Resistance through Down – Regulation of sp1-Dependent expression of the cadmium Transporter ZIP8 , J.Mol .pharm .74 (3) : 823-833 .
18. Garcia , S.F ., Navos ,D.A. ,Ramos ,P.M.C. and Belledone ,C. 2003 . Free and sol – gel Immobilized Alkaline phosphatase – based Biosensor for the Determination of pesticides and inorganic compounds, Ana . Chi .Act .484: 45-51.
19. Aanand , S., Purushothaman ,C.S ., Pal ,A.K. and Rajendran , K.V. 2009 . Toxicological studies on the effect of copper , lead and zinc on selected Enzymes in the Adductor Muscle and Intestinal Diverticula of the Green Mussel *pernaviridis* , J. Mar.Sci .39 (2) : 299-302 .

R.V.etal .2008 . Effect of chronic cadmium Exposure on Antioxidant Defense system in some tissues of rats : Protective Effect of Selenium , J. Physiol . Res .57 : 403-411.

20. Valko ,M .,Morris , H. and Cronin , M. T . 2005 . Metals ,Toxicity and oxidative stress , Current Medicinal Chemistery ,12 : 1187-1189 .
21. Ognjanovic , B.I , Markovic , S.D , pavlovic , S.Z, Zikic ,

دراسة التأثيرالسمي لمعقد الكادميوم (II) الجديد [CdL2] .1/2 H2O على فعالية انزيم GPT وALP لدى بعض اعضاء اناث الفئران بالمقارنة مع عقار السايكلوفوسفمايد المضاد للسرطان

ايمان هاشم عبد الرزاق*

*كلية العلوم للبنات - جامعة بغداد - قسم الكيمياء

الخلاصة :

من المعروف ان الكادميوم يضر بصحة الانسان وبشكل رئيسي من خلال تلوث مياه الشرب ، الاطعمة ، التبغ والملوثات الصناعية , الهدف من هذه الدراسة هو تقدير سمية معقد الكادميوم (II) الجديد: Bis[5-(p-nitrophenyl) -4`-phenyl-1,2.4- traizole-3-dithiocarbamato hydrazide] Cadmium (II) H ydra(0.5) ومقارنته مع عقار السايكلوفوسفمايد (CP) المضاد للسرطان في اناث الفئران نوع albino . لقد سبب هذا المعقد تغيرات في فعالية انزيمي ال GPT وال ALP في ثلاثة اعضاء بعد معاملة الحيوانات بجرع مختلفه هي (0.09,0.18,0.25) ملغم / فأر من المعقد الجديد لمدة ثلاثة ايام من خلال انخفاض غير معنوي (0.05) في فعالية انزيم GPT في الرئة ، الكبد ثم الكلية ، بينما حصلت زياده في فعالية انزيم ALP الموجود في الكلية ، الرئة و الكبد . **الاستنتاج** : بينت النتائج عدم وجود فروق معنوية ($P<0.05$) في فعالية انزيم GPT بين معاملة الفئران بمعقد الكادميوم (II) الجديد وعقار ال CP في الكبد والكلية وفعالية ALP في الكبد عند كل من الجرع الثلاث .