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A study of the effect of new cobalt (II) complex and cyclophosphamide drug on (GPT, ALP) activity by using *in vivo* system

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Abstract:

The present work involved a study the effect of cobalt(II) complex with formula [CoL(H2O)NO3] .4ETOH where L=Nitro [5-(P-nitro phenyl) -4-phenyl-1,2,4 traizole-3-dithiocarbamato hydrazide] aqua. (4) Ethanol and anti-cancer drug - cyclophosphamide on specific activity of two liver enzymes (GPT,ALP) by utilizing an in vivo system in female mice. On the enzymatic level an inhibition in the activity of GPT was noticed in different body organs such as liver, kidney and lung. The inhibition was noticed in both test and cyclophosphamide drug (cp). Mice were treated with three doses of cyclophosphamide (90,180, 250) µg/ mouse for three days. The same doses were used for the cobalt (II) complex. The liver shows the highest rate of(GPT) inhibition compared to other organs. The ratio was about 90% at three doses of cobalt (II) complex, this ratio was similar to ratio inhibition of cyclophosphamide at the same doses. On the contrary the enzyme ALP showed high activation in different organs such as liver, kidney and lung in both groups, test and cyclophosphamide drug (cp) at the three doses (90, 180, 250) µg /mouse. The result showed the highest ratio of activation in the kidney comparable with other organs. The maximum activation of cobalt(II) complex was about 1198% at a concentration 180µg/mouse.There are significant differences($P<0.05$) for two treatment when the concentration was increased.

Key words: Cyclophosphamide, GPT, Cobalt(II)complex , ALP, Anticancer drug.

Introduction:

The development of metal complexes with platinum central atoms such as cisplatin or carboplatin had an enormous impact on current cancer chemotherapy[1]. Preclinical and clinical investigations showed that the development of new agents with modes of action different from cisplatin is possible, thus complexes with iron, cobalt or gold central atom [2]. Cobalt-alkyne complexes represent a new class of antiproliferative drugs with high activity on cell lines derived from human solid tumors[3].Cobalt and chrom(II) ions could induce damages to proteins macrophage-H like cells in vitro[4]. Several alkyne cobalt carbonyl complexes inhabited the growth of human melanoma and lung carcinoma cell lines[5] .Roth et al [6] studied the cytotoxic activity of cobalt (III) complex, cis [Co (bpy) (2) C(II) H (23) NH (2) Cl)][2+] [1+] on HBL-100 human breast cancer cells, the cells succumbed to apoptosis (programmed cell death) as seen in the

change in the nuclear morphology and cytoplasmic features. Cyclopho-sphamide a nitrogen mustard, is an alkylating agent from the oxazophosphorine group [7]. It is widely used chemotherapeutic agent, it undergos extensive metabolism via the cytochrome P450 enzymatic system with phosphamide mustard and acrolein as the main active and inactive metabolites [8].Previous studies had shown that this compound is relatively inactive in vitro and is converted to the active form *in vivo*, trials in various tumor – bearing animals confirmed this in vivo activity and demonstrated fairly potent antitumor effect [9]. Enzymes are necessary for normal cellular metabolism including that of the liver, and the degenerative changes due to the combined metal toxicity exhibited in the liver alter the level of a number of its enzymes [10]. Glutamate–pyruvate transaminase (GPT) and alkaline Phosphatase (ALP) are released in acute and chronic liver disorders, these enzymes are biomarkers of acute hepatic damage [11].

Materials and Methods:

1.The animals

Eighty week female Balb /C mice(weight 30g) were divided into three groups, each group include nine mice as follows: Group (1), mice were given cobalt (II) complex (90, 180, 250) µg/ mouse. Group (2), mice were given cyclophosphamide (cp) at the same concentration (90, 180, 250) µg /mouse Group (3), was a control group (untreated). All groups were injected via intra peritoneal (I. p) on the first day. After 3 days of the experimental period, all the animals were killed by cervical decapitation [12]. Livers, kidneys and lungs were removed from each group then used for estimating Glutamate- pyruvate transaminase (GPT) and alkaline phasphatase (ALP) activity

2. Cyclophosphamide (cp) drug

The anti-cancer drug was provided by Baxter (Germany) (200 mg/10 ml).We prepared from this stock solution another solution by concentration 2.7mg⁄ 7.5 ml(normal saline)and then three concentrations were prepared from this solution (90,180,250)µg⁄mouse.

3. Cobalt (II) complex.

The new complex was prepared by Carolion, S.H in college of science for women – chemistry department [13].The complex was prepared by dissolving 200 mg in 10ml of normal saline (stock solution) and we prepared from this stock solution another solution by concentration 2.7mg /7.5ml(normal solution) and then three concentrations were prepared from this solution (90, 180, 250) µg/mouse.

4. Tissues collection (liver, kidney and lung)

The sample was collected from sacrificed animal using an Eppendrof tubes containing normal saline. Three treated animals and three untreated (control) were used for this purpose and the samples were stored at(- 20 C°) until processing .

5.Tissue homogenization and sample preparation

After the organs of animals were collected , the samples were prepared according to the method of Jenan [14]. Then 80% was extracted from the total activity of enzyme .We mixed the dry sand with prepared tissues for extraction.

Each tissue (liver, kidney and lung) was homogenizer with equal quantity of dry sand and mixed 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 Butanol : tissue (1:1) was added with mixing for 10 min .The tubes were

centrifuged at 3700 rpm for 10 min and the supernatant was taken which contain the enzymatic extract.

- GPT and ALP activity assay

The activity of Glutamate –pyruvate transaminase (GPT) was determined in liver, kidney and lung cells according to the method of Reitman *et al.,* [15]. Alkaline phosphatase (ALP) activity was determined in liver, kidney and lung cells according to the method of Bowers [16].

- Protein determination

The protein content in the samples was determined according to the method of Henry[17]. Using 0.5 gm /100ml bovine serum albumin (Bitest – Germany) as the standard solution.

6. Statistical analysis

Data was analyzed by 2-way analysis of variance with ANOVA – test.Data are presented as mean ± SD. The level of $p < 0.05$ was used as a significant for analysis of variance test (ANOVA)[18].

Results and Discussion:

1- Study of the GPT activity in different organs of female mice

- Liver

Table (1) showed the effect of cobalt (Π) complex and cyclophosphamide (cp) anti-cancer drug on GPT activity comparable with the control group. The mean value of GPT activity of the control group by U/mg proteins were reached to (27.2, 25.4, 26.8) respectively at three concentrations (90, 180, 250) µg/mouse. The results presented an evidence that treated with cobalt (Π) complex showed an inhibition in the activity of GPT at three concentrations (90, 180, 250) µg/mouse with highly significant ($p<0.05$), the inhibition ratio were about 95.83%, 82.41%, and 91.90% comparable with the control. The results also showed the inhibition of GPT specific activity at three doses when the female mice treated with anticancer drug (cp) comparison with control group. The results not to be found any significant differences between two treatment at these concentrations.

- Kidney

The results in table (2) showed the effect of cobalt (Π) complex on GPT inhibition specific activity. The highest ratio of inhibition was about 89.4% at concentration 90 µg/mouse compared to 90.9% inhibition ratio by using cyclophosphamide. As shown in the same table the effect of cyclophosphamide on GPT activity was similar to the effect of cobalt (Π) complex at two on concentrations (90,250) µg/mouse, there was no significant differences between them.

-Lung

The data of GPT- specific activity of lung from mice treated with cobalt complex and their treated with cyclophosphamide comparable with the control group are summarized in table (3). The results presented an evidence that treated with cobalt (Π) complex showed the inhibition ratio in enzymatic activity at three concentrations with highly significant ($P<0.05$) when the concentrations was increased in comparison with control. The inhibition rates were reached to (94.74%, 81%, 56%, 97.20%) respectively at three concentrations (90,180, 250)µg/ mouse. The statistical results showed the effect of cyclophosphamide in all concentrations in significant differences ($P< 0.05$) by the inhibition of GPT activity.

Table (1): The effect of cobalt (II) complex and cyclophosphamide on GPT (Glutamate pyruvate transaminase) specific activity of liver female mice in comparison with normal control

Conc.µg/ mouse	GPT specific activity by U /mg protein $\times 10^{-2}$ (mean± standard deviation			
Groups	90	180	250	Inhibition rate of Cobalt(II) complex comparison with normal control
Control	A, a 27.2±0.0022	A, a 25.4±0.0022	A, a 26.8±0.0022	%95.84
CP	B, a 3.0±0.0351	B ,a 2.57±0.0070	B, a 1.87±0.oo201	%82.41
Cobalt (II)complex	B, a 1.13±0.00321	B, b 4.467±0.007	B, c 2.170.00304	%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 rows.

Table (2):The effect of cobalt (II) complex and cyclophosphamide on GPT (Glutamate - pyruvate transaminase) specific activity of kidney female mice in comparison with normal control

Conc. µg/ mouse	GPT specific activity by U /mg protein $\times 10^{-2}$ (mean± standard deviation)			
Group	90	180	250	Inhibition rate of cobalt (II) complex comparison with normal control
Control	A, a 1.38±0.0021	A, a 1.36±0.0021	A, a 1.32±0.0021	%89.4
CP	B, a 0.125±0.004	B, a 0.273±0.00097	B, a 0.21±0.000557	%57.94
Cobalt (II) complex	B, a 0.146±0.0033	B, b 0.64±0.0027	B, a 0.3530.000751	%73.32

- Differences A, B, C are significant (p<0.05) to comparison columns.
- Differences a, b, c are significant (p<0.05) to comparison rows.

Table (3): The effect of cobalt (II) complex and cyclophosphamide on GPT (Glutamate - pyruvate transaminase) specific activity of lung female mice in comparison with normal control

Conc.µg/ mouse	GPT specific activity by U /mg protein $\times 10^{-2}$ (mean± standard deviation)			
Group	90	180	250	Inhibition rate of cobalt (II)complex comparison with normal control
Control	A, a 0.31± 0.0020	A, a 0.30 ± 0.0021	A, a 0.29 ±0.0022	%94.74
CP	B, a 0.0133± 0.00021	B, b 0.0467± 0.00078	B ,b 0.056± 0.000195	%81.56
Cobalt (II) complex	B, a 0.0163± 0.00015	B, b 0.0553± 0.000176	B ,c 0.081± 0.00190	%97.20

- Differences A,B,C are significant (p<0.05) to comparison columns.
- Differences a,b,c are significant (p< 0.05) to comparison rows.

According to our results it was shown that the inhibition ratio of GPT activity when tissues were treated with a new cobalt (II) complex and anti -cancer drug (cp) comparable with the control group, could be attributed the accumulation of toxic substances in animal body would cause grievous injury in hepatic tissue, and then would cause animal hepatase activity changes [19]. The enzymatic activity changes of liver major enzymes also reflect the damage degree of animal liver. A lot of GPT and GOT in liver will be pass into blood plasma, therefore the activation of liver aminotransferase will be decrease when organism is in an intoxication [20]. On the other hand, the dithiocarbamato ligand was anti–neoplastic activity were reported to induce apoptosis[21] .The results also showed the effect of cyclophosphamide (cp) was similar to the effect of cobalt (II) complex, we suggest the anticancer drug (cp) have inhibition effect due the drug cross-link DNA by adding alkyl group to the guanine base of DNA at N=7 positions of the imidazol ring that induce inhibition of DNA replication leading to cell death[7].

2. study of the (ALP) activity different organs of female mice

- Liver

The statistical results in table (4) shows the mean value of ALP specific activity of liver from mice after treated with cobalt (II) complex and their cyclophosphamide anti–cancer drug comparable with the control .The mean value of ALP enzyme specific activity of control group by U/mg protein were reached to (0.48, 0.47, 0.45) respectively at three concentrations (90, 180, 250) µg/mouse. The results showed a relative activation in both groups, cobalt (II) complex and cyclophosphamide (cp) at three doses with highly significant (p<0.05). the maximum activation was about %217

at a concentration 180 µg/mouse in the group treated with cobalt (II) complex. While it reaches a ratio of 289% in the group treated with cyclophosphamide in the same concentration. There were no significant differences between two treatment at two concentrations (180, 250) µg/ mouse.

- kidney

As shown in table (5) the kidney alkaline phosphatase enzyme showed a relative activation in both groups, cobalt (II) complex and cyclophosphamide (cp) at three concentrations comparable with the control group. The kidney showed the highest ratio of activation compared to other organs, with highly significant ($p<0.05$). The results also showed there was significant differences ($p<0.05$) with concentrations increased when the female mice treated with anticancer drug comparable with control. The effect of cobalt (II) complex was similar to the effect of cyclophosphamide at two concentrations (90, 250) µg/ mouse .

-Lung

The data of ALP- specific activity of lung from mice treated with cobalt (II) complex and their treated with cyclophosphamide comparable with the control group are summarized in table (6). The enzymatic concentration shows an activation in the activity of ALP at three concentrations. The results also showed the effect of two treatments in elevation of ALP activity with the increased concentration. As shown in table (6) the combined effect of cobalt (II) complex and cyclophosphamide at two concentrations (90, 250) µg/ mouse. It was found no significant between them. The maximum activation of ALP activity for the two treatments was at a concetration180 µg/ mouse.

Table(4): The effect of cobalt (II) complex and cyclophosphamide on Alkaline phosphatase (ALP) activity of liver female mice in comparison with normal control group

Conc. µ g/ Mouse / Groups	ALP specific activity by U /mg protein $\times 10^{-2}$ (mean ± standard deviation)		
	90	180	250
Control	A ,a 0.48 ± 0.00090	A ,a 0.47± 0.0009	A, a 0.450.0009
CP	C, a 1.03 ± 0.0038	B, a 1.83± 0.0095	B ,a 1.0 ± 0.0032
Cobalt (II) complex	B, a 0.78 ± 0.0017	B, b 1.49 ± 0.00186	B, a 1.02± 0.0015

- Differences A, B, C are significant ($p<0.05$) to comparison columns.
- Differences a, b, c are significant ($p< 0.05$) to comparison rows.

Table(5): The effect of cobalt (II) complex and cyclophosphamide on ALP (Alkalinephospatase) specific activity of kidney female mice in comparison with normal control

Conc.µg/ mouse / Groups	ALP specific activity by U /mg protein $\times 10^{-2}$ (mean ± standard deviation)		
	90	180	250
Control	A ,a 0.138± 0.00099	A, a 0.136 ± 0.0020	A, a 0.139 ± 0.002
CP	B, a 1.26 ± 0.0032	C, b 2.773± 0.020	B,a 1.270 ± 0.022
Cobalt (II) complex	B, a 1.0 ± 0.0035	B, b 1.766 ± 0.026	B, b 1.486± 0.053

- Differences A, B, C are significant ($p<0.05$) to comparison columns.
- Differences a, b, c are significant ($p<0.0\ 5$) to comparison rows.

Table(6): The effect of cobalt (II) complex and cyclophosphamide on ALP (Alkaline phosphatase) specific activity of lung female mice comparison with normal control group.

Conc . µg/ mouse / Groups	ALP specific activity by U/mg protein $\times 10^{-2}$ (mean ± standard deviation)		
	90	180	250
Control	A ,a 0.038 ± 0.000030	A, a 0.040 ± 0.000030	A, a 0.039 ±0.0003
CP	B, a 0.77 ± 0.0023	C,b 2.30 ±0.00063	B,c 1.15 ± 0.0030
Cobalt(II) complex	B, a 0.80 ± 0.0014	B, b 1.55 ± 0.0060	B, c 1.30± 0.0020

- 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 results obtained in this study, indicated that the activities of alkaline

phosphatase was significantly increased comparable with the control. It could be attributed to the hepatic damage resulting increased release and leakage out of this enzyme from the liver cytosol in to the blood stream which gives an indication on the hepatotoxic effect of this metal [21]. Rotimi *et al*., [22] which found the cobalt ion (Co^{+2}) a better activator of rat kidney ALP, the activation may be through formation of an activated (Co^{+2}) ALP complex where (Co^{+2}) occupies both catalytic and structural sites of alkaline phosphatase. The new cobalt (II) complex was similar effect to cyclophosphamide (cp) that could be attributed to the cp exhibit greates cytotoxicity against cell actively replicating .The DNA as umpiring of DNA strands at this stage makes the nucleotide residues more susceptable to alkylation, hepatic activation of cp leading to the formation of toxic metabolites caused damage to the liver tissues as shown by increased ALP [8].

Conclusion: The study showed the cobalt (II) complex have a cytotoxic effect by reducing the GPT activity and activation of ALP activity at different concentrations these effect were similar the effect of cp at the same doses .

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دراسة تأثير معقد الكوبلت (II) الجديد وعقار السايكلوفوسفومايد على فعالية أنزيمي (ALP , GPT) باستخدام نظام داخل جسم الكائن الحي *in vivo*

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الخلاصة :

تم دراسة تأثير معقد الكوبلت (II) بصيغته [CoL(H2O)NO3]. 4ETOH ، حيث يكون الليكاند Lبالمعقد هو نايترو [5- (بارا- نايتروفنيل)-4 فنيل – 1،2، 4، ترايزول 3- ثنائي كاربميت- هيدرازين] .جزيئة ماء.ايثانول (4) وعقار السايكلوفوسفومايد المضاد للسرطان على الفعالية النوعية لأنزيمي الكبد (GPT،ALP) باستخدام نظام داخل الجسم الكائن الحي *in vivo* لإناث الفئران المختبرية. لقد لوحظ على المستوى الانزيمي حدوث تثبيط في فعالية انزيم الـGPT في مختلف الأعضاء المستخدمة مثل الكبد والكلية و الرئة، وقد شمل التثبيط كلا المجموعتين المعاملة بمعقد الكوبلت(II) وعقار السايكلوفوسفومايد وذلك عند معاملة الفئران بثلاث تراكيز للسايكلوفوسفومايد هي (90، 180، 250) مايكروغرام/ للفأر ولمدة ثلاث ايام وقد استخدمت التراكيز نفسها للفئران المعاملة بمعقد الكوبلت (II) الجديد. وقد سجلت النتائج أعلى مستوى للتثبيط في الكبد مقارنة مع الأعضاء الأخرى، حيث بلغ معدل نسبة التثبيط حوالي 90% للجرع الثلاث المستخدمة لمعقد الكوبلت (II) وهي مشابهة لمعدل نسبة التثبيط في فعالية الـ GPT لعقارالسايكلوفوسفومايد وبالجرع الثلاث نفسها.من ناحية أخرى فقد لوحظ أن هناك تنشيطاً في فعالية أنزيم الـِ ALPفي الأعضاء المختلفة مثل الكبد، الكلية والرئة للمجموعتين المعاملة بمعقد الكوبلت(II) والمجموعة المعاملة بعقار السايكلوفوسفومايد وبالتراكيز الثلاث المستخدمة (250,180,90) مايكروغرام/للفأر. وقد بينت النتائج أن مستوى التثبيط في فعالية أنزيم ALPفي الكلية أعلى من الأعضاء الأخرى، حيث بلغت نسبة التثبيط 1198% لمعقد الكوبلت عند التركيز180 مايكروغرام/للفأر. كما أظهرت النتائج وجود فروق معنوية($P<0.05$) للمجموعتين المعاملين مع زيادة التركيز.

الكلمات المفتاحية:السايكلوفوسفومايد،GPT، معقد الكوبلت(II)، ALP، العقار المضاد للسرطان.