Antioxidant Activity and Histopathological Examination of Chromium and Cobalt Complexes of Bromobenzaldehydeiminacetophenone Against Ehrlich Ascites Carcinoma Cells Induced in Mice

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ABSTRACT

The continuous demand for new chemotherapeutic agents indicates that new approaches are critically needed. The current study was under taken to examine the effect of the two novel antitumor complexes namely cobalt and chromium complexes of bis-(4-bromobenzaldehydeiminoacetophenone), BBIA-Co and BBIA-Cr, on liver, kidney and heart of Ehrlich ascites carcinoma (EAC) bearing male *albino* mice. The effect of the two complexes on antioxidant status of the animals and histopathological examination of liver, kidney and heart tissues were also examined. Results indicated that treatment with either BBIA-Co or BBIA-Cr had ameliorated to some extent the changes in liver function exerted by EAC inoculation. Both complexes had no significant effect on kidney function, while they induced cardiac toxicity to animals. The study also showed no significant changes in the antioxidant status of EAC inoculated mice treated with BBIA-Co compared to normal animals, which was not indicated after BBIA-Cr treatment. The biochemical data results were further supported by histopathological examination.

Key Words: Cobalt and chromium complexes; Ehrlich ascites carcinoma; Antioxidant activity; Histopathology.

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INTRODUCTION

Cancer cells have higher levels of reactive oxygen species (ROS) than normal cells, resulting in increased oxidative stress. The latter may result in injury of cellular components causing cell death. Several enzymatic and non-enzymatic components, available in animal's body, form a major defense mechanism against oxidative stress [1]. An antioxidant is a substance that delays, prevents or removes oxidative damage. Among the antioxidant enzymes are superoxide dismutase (SOD) and catalase (Cat) which convert active oxygen molecule into nontoxic ones.

The implication of free radicals in tumor is well known [2], [3]. Oxidative mechanisms have a role in the initiation, promotion and progression of carcinogenesis [4], [5]. The increased oxidative stress in cancer can be due to the increased formation of ROS with normal defense
mechanism. It might be also due to decreased defense mechanism or failure in repair of oxidative damage [6]. The most common approach to measure oxidative stress is to measure its derivatives or end products, such as lipid peroxidation products. Indirect methods

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include measurement of antioxidant levels or total antioxidant status [7].

Most drugs used for cancer treatment are known to affect many vital organs such as liver kidney and heart. For example, cisplatin, one of the metal complexes widely used for cancer treatment, induces tissue toxicity, in particular to the kidney (Giaccone, 2000). The undesirable side effects of most of the available chemotherapeutic agents encouraged continuous research for new reagents that exhibit anti-tumor activity. We have recently reported the existence of in vivo antitumor activity for both Co and Cr complexes of bis-(4-bromobenzaldehyde-iminoacetophenone), BBIA-Co and BBIA-Cr, against Ehrlich ascites carcinoma cells (EAC) induced in mice [8], [9]. We aimed in the current study to explore the biological functions of some of the vital organs including liver, kidney and heart of EAC bearing mice post treatment with either of the two complexes. Furthermore, evaluating the antioxidant status of EAC bearing mice under treatment and examining the histopathological changes in liver, kidney and heart tissues of treated animals were carried out.

MATERIALS AND METHODS

Materials

Synthesis of the complexes:The BBIA-Co and BBIA-Cr complexes were prepared according to the method described by Ramadan et al., (2014).

EAC cells:Cells were obtained from American Type Tissue Culture Collection, Manassas, VA, USA.

Vehicle:The BBIA-Co and BBIA-Cr complexes were freshly dissolved, directly before use, in a vehicle containing dimethyl sulfoxide (DMSO) and distilled H2O (4:1, v/v).

Methods

a- Animals Management and Groups: 144 Male Swissalbino mice (30-33 g body weight) were enrolled in the study. Animals were kept for one week acclimatization period under controlled conditions of temperature, humidity and light/dark cycle (23- 25 ºC, 50-55% and 12 h L/12h D, respectively). Mice were divided randomly and equally into six main groups. Normal group:Included healthy animals ip. injected with 0.2 ml saline, 3 times/week for 2 weeks. EAC group: Animals in this group were ip. inoculated with 2x106 Ehrlich ascites carcinoma cells/mouse and were monitored for 14 days. BBIA-Co group: In this group, healthy animals were ip. injected with 0.2 ml of freshly dissolved Co complex at a final dose equivalent to 40 mg of BBIA-Co/kg body weight (representing 10% of the lethal dose for BBIA-Co), 3 times/week for 2 weeks. BBIA-Cr group: In this group, healthy animals were ip. injected with 0.2 ml of freshly dissolved Cr complex at a final dose equivalent to 70 mg of BBIA-Cr/ kg body weight (representing 10% of the lethal dose for BBIA-Cr), 3 times/week for 2 weeks. BBIA-Co treated group: Animals were ip. inoculated with EAC cells (2x106 cells/mouse) followed, after 24h, by daily injection of 40 mg BBIA-Co/Kg body weight dissolved in 0.2 ml vehicle, 3 times/week for 2 weeks. BBIA-Cr treated group: Animals were ip. inoculated with EAC cells (2x106 cells/mouse) followed, after 24 h, by daily injection of 70 mg BBIA-Cr/Kg body weight dissolved in 0.2 ml vehicle, 3 times/week for 2 weeks. Animals were monitored regularly for alterations in body weight, for the development of any signs of toxicity and mortality.

b- Biochemical Assays:

i- Effect of BBIA-Co and BBIA-Cr on liver, kidney and heart functions:Blood samples withdrawn from four mice in equal amounts were pooled on a clean tube; resulting in six samples in each group; to obtain sufficient quantity of serum to determine liver, kidney and heart functions. Liver function included activities of alanine amino transeferase (ALT) and aspartate amino transferase (AST) as well as levels of total proteins (TP), albumin and globulin. Kidney function was evaluated by determining levels of urea and creatinine. Lactase dehydrogenase (LDH), creatine kinase (CK) and CK-MB activities were determined and were used as markers for evaluating heart function.

ii- Effect of BBIA-Co and BBIA-Cr on antioxidant activities:Blood samples withdrawn from retro-orbital sinus of the mice on heparin tubes were used for determination of antioxidant markers including: total antioxidant capacity, malondialdehyde (MD), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (Cat).

c- Effect of BBIA-Co and BBIA-Cr on liver, kidney and heart tissue:Liver, kidney and heart were prepared for histological examination by Heamatoxylin and Eosin stain according to the method described by Drury and Wallington (1980).

d- Statistical analysis: Statistical analysis was performed using SPSS 24.0 for windows (SPSS Inc, USA). Descriptive statistics are shown as mean ± standard error of the mean. P values smaller than 0.05 were considered statistically significant.

RESULTS

A. Effect of the cobalt and chromium complexes on liver, kidney and heart functions:

Results indicated significant variations in all liver function parameters (ALT, AST, total proteins, albumin and globulin) in mice bearing EAC cells. Administration of either Co or Cr complex to tumor bearing mice ameliorated to some extent the changes in liver parameters, mainly total proteins and albumin (Table 1). No significant changes in kidney function parameters (serum urea and creatinine) were observed in EAC bearing tumor group or post administration of the complexes under examination. (Table 1).

Results also illustrated significant elevation in LDH activity and cardiac markers in EAC bearing mice

compared to normal mice group. Post treatment with either Co complex or Cr complex had significantly decreased LDH activity. Moreover, compared to normal mice, elevated cardiac markers were evident post BBIA-Co and BBIA-Cr treatment (Table 1).

Table 1: Effect of BBIA-Co and BBIA-Cr complexes on liver, kidney and heart profile.

Groups Parameters	Normal	EAC	BBIA-Co	BBIA-Cr	BBIA-Co treated	BBIA-Cr treated
ALT (U/L) *P*	29.4± 1.83	50.4 ± 2.27 0.001	54.1 ± 4.60 0.001 (NS)	43.5 ± 4.48 0.02 (NS)	58.2 ± 5.06 0.001 (NS)	35.8 ± 2.30 0.03 (0.001)
AST (U/L) *P*	57.5 ± 3.22	102.7±10.89 0.001	98.5 ± 2.29 0.001 (NS)	77.0 ± 6.12 0.04 (0.04)	104.7 ± 1.73 0.001 (NS)	183.0 ± 5.74 0.001(0.001)
TP (mg/dl) *P*	4.6 ± 0.03	3.6 ± 0.01 0.001	4.2 ± 0.04 0.001(0.001)	4.4 ± 0.05 0.02(0.001)	4.3 ± 0.04 0.001(0.001)	4.4 ± 0.10 0.01(0.001)
Albumin(mg/dl) *P*	2.1 ± 0.07	1.6 ± 0.01 0. 001	1.9 ± 0.03 NS (0.001)	2.1 ± 0.04 NS (0.001)	2.1 ± 0.02 NS (0.001)	2.0 ± 0.05 NS (0.001)
Globulin (mg/dl) *P*	2.5 ± 0.08	1.9 ± 0.001 0.001	2.3 ± 0.04 NS (0.001)	2.4 ± 0.08 NS (0.001)	2.1 ± 0.04 0.01 (0.001)	2.3 ± 0.08 NS (0.001)
Urea (mg/ dl) *P*	72.0± 3.24	70.2 ± 1.15 NS	63.3 ± 2.61 0.03 (0.02)	59.4 ± 2.82 0.001(0.001)	59.0 ± 1.68 0.001(0.001)	63.8 ± 3.83 NS (0.01)
Creatinine(mg/dl) *P*	0.3 ± 0.01	0.3 ± 0.01 NS	0.3 ± 0.01 NS	0.3 ± 0. 01 NS	0.3 ± 0.01 NS	0.3 ± 0.01 NS
LDH (U/L) *P*	258.6±23.98	455.2±26.95 0.01	339.8±23.43 0.05 (0.01)	323.9±16.81 NS (0.001)	346.9 ± 14.16 0.04 (0.02)	384.2 ± 15.37 0.001 (0.01)
CK (U/L) *P*	94.2 ± 18.42	130.2 ± 1.53 0.01	147.0±15.93 0.01 (NS)	163.9±25.76 0.03 (NS)	169.6 ± 17.86 0.03 (0.01)	118.5 ± 13.34 NS (NS)
CK-MB (U/L) *P*	44.8 ± 3.80	65.9 ± 1.45 0.001	85.0 ± 3.34 0.001(0.001)	74.7 ± 5.71 0.001 (NS)	79.1 ± 5.41 0.001 (0.01)	56.2 ± 4.50 NS (NS)

P: numbers outside parentheses give P value compared to normal group; P values inside parentheses give P value compared to EAC group; P ≤ 0.05 is significant; NS: non-significant.

. Effect of the cobalt and chromium complexes on antioxidant activities:

Significant changes in antioxidant parameters were detected in EAC bearing mice, where MDA, as well as studied antioxidant enzymes, except SOD, showed remarkable increase in their mean values compared to normal healthy mice. Tumor bearing mice treated with the Co complex had almost comparable mean, to normal mice, for TAC, SOD, GR and Cat, while MDA was still higher than normal mice. On the other hand, post-treatment with the Cr complex did not ameliorate the changes appeared in antioxidant status post inoculation of EAC cells to mice, whereTAC, MDA, SOD and Cat showed higher mean compared to normal mice group. The current study revealed significantly elevated MDA level, GR and Cat activities when the Cr complex was injected to healthy mice. Moreover, the Co complex injection to normal mice did not induce significant change in antioxidant parameters compared to normal mice group (Table 2).

C. Effect of BBIA-Co and BBIA-Cr complexes on liver, kidney and heart tissue:

Histopathological examination of liver tissue from normal, EAC, BBIA-Co, BBIA-Cr and treated groups is illustrated in Figure 1. Histological examinations for kidney and heart tissues for all groups are indicated in Figures 2 and 3, respectively.

Table 2: Effect of BBIA-Co and BBIA-Cr complexes on antioxidant parameters.

Groups Parameters	Normal	EAC	BBIA-Co	BBIA-Cr	BBIA-Co treated	BBIA-Cr treated
TAC (mmol/L) *P*	1.35 ± 0.06	0.56 ± 0.03 0.001	0.75 ± 0.16 NS (NS)	1.20 ± 0.21 NS (NS)	1.12 ± 0.14 NS (NS)	1.59 ± 0.02 0.001 (0.001)
MDA (nmol/L) *P*	11.70± 1.18	16.70±0.82 0.04	12.48± 2.34 NS (NS)	23.26± 3.05 0.001(0.02)	15.94 ± 0.80 0.001 (NS)	18.19 ± 1.91 0.01 (NS)
SOD×10^3(U/ml) *P*	2.17 ± 0.13	2.37 ± 0.27 NS	1.87 ± 0.12 0.02 (0.04)	2.71 ± 0.41 NS (NS)	2.59 ± 0.43 NS (NS)	2.98 ± 0.30 0.03 (0.05)
GR (U/L) *P*	13.44±0.42	15.48±0.53 0.01	12.98 ± 0.28 NS (0.001)	17.42 ± 0.96 0.001 (NS)	14.52 ± 0.79 NS (NS)	14.28 ± 0.55 NS (0.001)
Cat × 10^3 (U/L) *P*	0.25 ± 0.03	0.41 ± 0.05 0.001	0.32 ± 0.04 NS (0.02)	0.45 ± 0.05 0.001 (NS)	0.36 ± 0.04 NS (NS)	0.45 ± 0.04 0.001 (NS)

P: numbers outside parentheses give P value compared to normal group; P values inside parentheses give P value compared to EAC group; P ≤ 0.05 is significant; NS: non-significant.

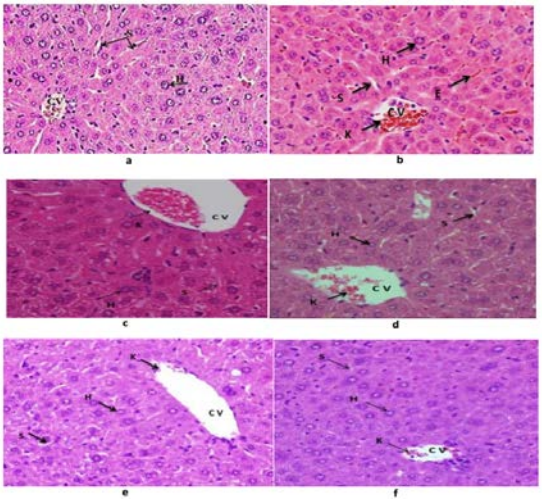

Figure 1: Histopathology of liver tissue (H&E X 40) of: a (normal group), b (EAC group), c (Co- complex group), d (Cr- complex group), e (Co-complex treated group), f (Cr- complex treated group)

CV: central vein, H: hepatocytes, S: blood sinusoids, K: kupffer cell, E: hemorrhage

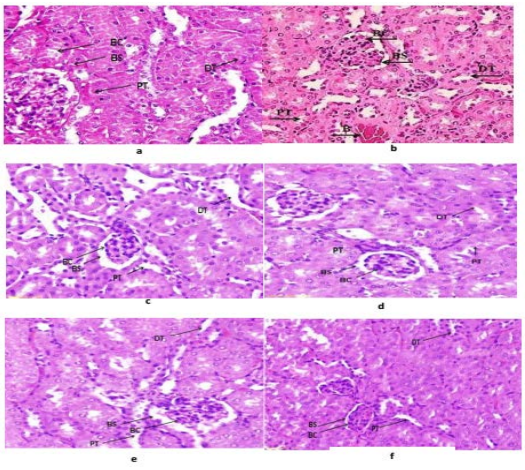

Figure 2: Histopathology of kidney tissue (H&E X 40) of: a (normal group), b (EAC group), c (Co-complex group), d (Cr- complex group), e (Co-complex treated group), f (Cr- complex treated group)

BC: Bowman's capsule, BS: Bowman's space, PT: proximal tubule, DT: distal tubuleB: blood vessels congestion

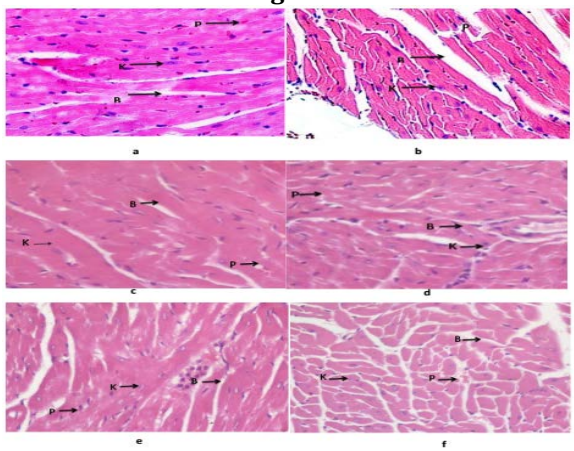

Figure 3: Photomicrograph of cardiac tissue (H&E X 40) of: a (normal group), b (EAC group), c (Co-complex group), d (Cr- complex group), e (Co-complex treated group), f (Cr- complex treated group)

P: pyknosis, K: karyolysis, B: blood vessel.

DISCUSSION

Recently, we have reported that the two metal complexes namely cobalt and chromium bromobenzaldehydeiminoacetophenone (BBIA-Co & BBIA-Cr) exhibited in vivo anti-tumor activity against EAC cells [8], [9]. Both two complexes have resulted in life span prolongation of EAC bearing mice, decreased ascetic fluid accumulation and decreased EAC viability. However, the Co complex showed greater antitumor activity compared to the Cr one. This was evident from the reported results which indicated higher mean survival time, prolonged life span as well as deceased cell viability post BBIA-Co treatment [8], compared to post BBIA-Cr treatment [9]. In this study, we extended our investigation to evaluate the effect of the two complexes on the activity of some of the vital organs. The antioxidant capacity of the animal bodies post treatment with the complexes were also evaluated. The histopathological changes associated with treating EAC bearing animals were also examined.

In the current study, liver function was found to be significantly affected post EAC inoculation. Compared to normal mice group, elevated serum ALT and AST activities and decreased plasma proteins were observed in EAC bearing mice. These results were further supported by histopathological examination of liver tissue from EAC bearing mice indicating increased number of Kupffer cells, congested central vein with hemorrhage and dilated congested blood sinusoids. Restoration of liver function was noted following treatment by the complexes, where remarkable increase in plasma proteins towards normal values was detected. However, treatment did not ameliorate the elevated liver enzymes. Furthermore, injecting the complexes to healthy animals (BBIA-Co or BBIA-Cr groups) had resulted in significant increase in ALT and AST activities without affecting plasma proteins. The increased AST (and to a lesser extent ALT) activity in the four groups of animals receiving the complexes under investigation might indicate pathology in other organs, or the existence of other sources for these enzymes, probably muscle source, as cardiac muscle [10]. Histopathological examination of liver tissue from treated EAC bearing mice indicated normal liver tissue, which in turn supports restoration of liver activity following treatment, and consequently points out to the potential activity of the drug against liver damage induced by EAC cells inoculation.

In the present work, unchanged kidney function by EAC inoculation was noted. No significant differences in serum urea or creatinine levels were obtained between EAC and normal control mice groups. Injection of both BBIA-Co and BBIA-Cr complexes were safe on kidney and did not affect the kidney function. Further support for these biochemical data of kidney function was carried out by histopathological examination of renal tissue. The examination indicated no significant alteration in kidney tissue from EAC, treated EAC bearing tumor, BBIA-Co and BBIA-Cr groups.

Tumor, on the other hand, had resulted in significant deteriorations in cardiac function characterized by increased cardiac enzymes (LDH, CK and CK-MB) as well as elevated transaminases; in particular AST. In cancer, LDH is mainly produced during anaerobic pathway of glycolysis [11]. Many diseases induce elevation in the levels of LDH isoenzyme. The elevated LDH in EAC bearing mice in this study could be due to hepatotoxicity, cardiac diseases and/or fast tumor cells metabolism. Histopathological examination of heart tissue of EAC bearing mice indicated dilated spaces between myocyte cells, nuclear myocardial pyknosis,

karyolysis and wide dilated blood vessels with few inflammatory cells. Treatment with either BBIA-Co or BBIA-Cr complex had significantly reduced LDH activity, although CK, CK-MB and AST were higher post Cobalt complex administration than untreated EAC bearing mice. It is worth to indicate that injection of either complex to healthy animals significantly raised cardiac parameters, suggesting that the complexes may induce cardio-toxic effect to mice. It is worth noting to indicate that cardiotoxicity was greater in mice receiving Co complex, either healthy mice or EAC cells inoculated mice, than those receiving Cr complex. Histopathological study of heart tissue of animal (either healthy or inoculated with EAC cells) injected with Co or Cr complex further supported the cardiotoxic effect of the complexes.

The EAC cells inoculated to mice had resulted in significant reduction in the total antioxidant capacity of the animal body. Furthermore, all markers used for examining the oxidative capacity (malondialdehyde level and glutathione reductase and catalase activities), except for SOD, were elevated in tumor bearing mice compared to healthy animals. Oxidative mechanisms have a role in the initiation, promotion and progression of carcinogenesis [4], [5]. Oxidative stress in cancer was suggested to be due to many factors. It might be due to increased formation of ROS when the antioxidative defense mechanism works normally. It could be also due to the decreased antioxidant defense mechanism with unchanged status of exposure to ROS. Furthermore, failure in repair of oxidative damage, which leads to the increased presence of ROS. Finally, it might be a combination of all the above [6].

The available literature regarding the relationship between cancer and activities of antioxidant enzymes are characterized by contradiction and inconsistency. While most studies [12], [13] observed decreased antioxidant enzymes activities in cancer, others indicated elevated activities in cancer patients [14] Here, we suggest that increased catalase and glutathione reductase activities in EAC bearing mice could be due to increased production of ROS and an attempt of the cell to neutralize these free radicals by elevating antioxidant enzymes activities. However, there is no equilibrium between the rate of production and the rate of neutralization of these free radicals resulting in increased oxidative stress. The above suggestion was further supported by our finding indicating decreased total antioxidant capacity and elevated malonaldhyde levels of animals bearing tumor, since total antioxidant capacity is a measure of the whole body oxidative stress body [15], [16].

Post treatment of tumor bearing mice with the Co complex had significantly restored antioxidant statues of the animals to values comparable to control mean values, except malondialdehyde which showed significantly higher means. On the other hand, mice bearing tumor treated with the Cr complex showed significantly higher means for total antioxidant capacity, malondialdehyde, superoxide dismutase and catalase compared to their matched values in normal mice. Changes in antioxidant parameters in Cr complex treated mice bearing tumor could be due to both effect of the complex [17], [19] and/or the indirect effect of the tumor regression itself that induces free radicals [Ozkan&Fiskin, 2005].

It is worth mention to indicate that healthy animals injected with BBIA-Co complex had comparable antioxidant status to normal mice. Meanwhile, compared to normal mice, post injection of Cr complex to healthy mice had significantly increased malondialdehyde, glutathione reductase, and catalase mean values. These data emphasize the undesirable side effects of Cr complex on animal oxidant/antioxidant status, since the desirable treatment for malignant tumor would be an agent that has effect on tumor cells without causing side effects on healthy cells. The effect of Cr complex on antioxidant status of healthy animals could be due to its metabolic activity, where different authors indicated that many drugs used in cancer chemotherapy might form free radicals because of their metabolic activities [17], [19].

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