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Anticancer Activity of Bis (4-bromobenzaldehyde-4-iminacetophenone) Diaquozinc (II) Nitrate Complex against Ehrlich Ascites Carcinoma Cells Induced in Mice

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ABSTRACT

Many metal-containing compounds were utilized throughout history to treat a wide variety of disorders. Cancer is one of the major cause of death in the world. Most conventional anticancer drugs were designed with DNA synthesis as their target. The present study was carried out to investigate in vivo antitumoreactivity of bis (4-bromobenzaldehyde-4-iminacetophenone) diaquozinc (II) nitrate complex (BBIA- Zn) against Ehrlich Ascites carcinoma cells induced in male albino mice. In vitro, the complex showed cytotoxicity towards cancer cell lines. In vivo, the results indicated that the parameters of EAC bearing mice alone resulted in significant increase in ascetic fluid, liver, kidney, cardiac enzymes and lipid profile accompanied with reduced in cell viability and antioxidant activity when compared with control group. The complex lowered the tumor burden markedly in a concentration-dependent manner and antagonized the effects induced by EAC cells, towards the normal value of control. In conclusion, our results revealed that the complex has a potential role as an anticancer agent with minimal side effects. This may possibly via its redox activity, through its lipophilic nature and maybe there was covalent cross linking of DNA nitrogen nucleophiles.

INTRODUCTION

Cancer is a major public health problem worldwide with increasing incidence and mortality. The number of global cancer deaths is projected to increase 45% from 2007 to 2030 (from 7.9 million to 11.5 million deaths), influenced in part by an increasing and aging global population. The estimated rise takes into account expected slight decline in death rates for some cancers in high resource countries. New cases of cancer in the same period are estimated to jump from 11.3 million in 2007 to 15.5 million in 2030 (Kushi, L.H., *et al*., 2012). Cancer treatment is usually a combination of a number of different modalities. If the tumor is amenable to surgery, then surgery is the single most effective tool in the anticancer armamentarium. Targeted radiotherapy is another option, as are combinations of anticancer drugs. Most conventional anticancer drugs have been designed with DNA synthesis as their target (Göschl, S., *et al*., 2017). Transition metal based compounds constitute a discrete class of chemotherapeutics, widely used in the clinic as antitumor and antiviral agents. Examples of established antitumor metallodrugs, routinely used in clinic, are cisplatin [cis-diamminedichloro-platinum(II)] and its analogues (Jamieson, E.R. and S.J. Lippard, 2009). However, drug resistance and side effects have limited its clinical utility. These limitations have prompted a search for more effective and less toxic metal based antitumor

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agents (Brabes, V. and O. Novakova, 2006). Different metal like gallium, germanium, tin, bismuth, titanium,ruthenium, rhodium, iridium, molybdenum, copper and gold were shown effective against tumors in man and animals (Rafique, S., *et al*., 2010).

Zinc is one of the essential trace elements to animal body and it is nontoxic even at higher doses (Vallee, B.L. and K.H. Falchuk, 2007). Zinc is highly participated in protein, nucleic acid, carbohydrate and lipid metabolism as well as in the control of gene transcription and other fundamental biological processes. The homeostatic mechanism that regulate its entry into, distribution in and excretion from cells and tissues are sufficient resulting in its safety. The recommended daily allowance of zinc is approximately 15mg/day (Vallee, B.L and D.S. Auld, 2008). Zinc is a low coast, biocompatible metal and it is found in more than 300 enzymes in every biological cells. The curative role of zinc in wound healing and gastric ulcer (Andrews, M. and C. Gallagher-Allred, 2009) is well established. A large number of zinc (II) complexes show promising antimicrobial activities against bacterial strains and fungi (Amin, M., *et al*., 2009). The majority show in vitro in determine cytotoxicity against different human cancer cell lines including hepatocellular carcinoma (HepG2 and SK-Hep-1), human cervical (Hela), prostate (PC3), pancreatic and lung cancer cell lines (Failli, P., *et al*., 2009). The antimicrobial properties of metals have been recognized for centuries and have represented some of the most fundamental breakthroughs in medicinal history (Scozzafava, A., *et al*., 2001). Many studies stressed the role of metal ions in important biological processes, whereas the inorganic pharmacology started to be an important field with more than 25 inorganic compounds, being used in therapy as antibacterial, antiviral, and anticancer drugs. Kirschner*et al.* (2000), have suggested that the transfer of the metal ion from the ligand to the cancer-associated viruses was an important mechanism for designing new anticancer therapies. Experimental tumors have great importance for the purposes of modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest (Siems, W., 1993). It appeared firstly as a spontaneous breast cancer in a female mouse used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse. Loewenthal and Jahn (1932) obtained the liquid form in the peritoneum of the mouse and named it as "Ehrlich ascites carcinoma" due to the ascites liquid, together with the carcinoma cells. EAC is referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor specific transplantation antigen (Lettre, R., *et al*., 1992). All of these make EAC an efficial model of experimental tumor induced in animals. The compounds showed considerable cytotoxic activity in the trypan blue exclusion method. In vivo cancer model (Ehrlich ascitic carcinoma model), the compounds significantly ($P<0.05$) reversed the tumor-induced changes in the parameters monitored viz., percentage increase in body weight, percentage increase in lifespan, tumor-viable count, and hematological parameters (total WBC, total RBC, and Hemoglobin count) (Shirin, C.Z. and R.N. Mukherj, 2010). These effects were almost comparable to cisplatin—the standard drug used in the study. The compounds, however, were found to have good effect in prolonging the lifespan (ILS) as compared to standard drug cisplatin. These findings imply that the compounds might be having some anticancer principles (Devi, P.U., *et al*., 2005), the compounds had shown promising cytotoxic activity when screened using the in vitro method and at the same time were shown to have good activity when tested using the Ehrlich ascites carcinoma model (Eckhardt, A.E., *et al*., 2006). Further, the promising results were observed for the antimicrobial screening especially for the Co(II) complex against the fungi what may be attributed to the fact that the metal complexes are potentially active against fungal cells than bacterial cells (Garen, A. and C. Levinthal, 2001).

Recently, Ramadan *et al.*, (2015) showed that a novel bis (4-bromobenzaldehyde-4-iminacetophenone) diaquozinc (II) nitrate complex (BBIA- Zn) was synthesized and was characterized by elemental analysis, mass, IR and NMR spectrometry. The complex showed promising antimicrobial activity against gram positive and gram negative bacteria and fungi. The complex, also, exhibited strong in vitro cytotoxic activity against liver carcinoma cell line (HepG2, IC_{50} value = 5.1µg/ml) and tow breast cancer cell lines with IC_{50} = 3.9 µg/ml and 4.2µg/ml for MCF7 and T47D respectively. The present study was carried out to investigate in *vivo* antitumor activity of bis (4-bromobenzaldehyde-4-iminacetophenone) diaquozinc (II) nitrate complex against Ehrlich Ascites carcinoma cells induced in male *albino* mice.

MATERIALS AND METHODS

a- Preparation of BBIA- Zn complex:

The complex under investigation was prepared according to the method described by Ramadan *et al* (2015).

b- Biological activity:

In vitro antibacterial and antifungal activity of the ligand and the synthesized complexewase tested against the two bacteria: *Escherchia coli* as Gram-negative bacteria and *Stapylococcus aureus* as Gram-positive bacteria, and the two fungus: *Aspergillus flavus* and *Candida albicans*. The tests were carried out using paper disk diffusion method. The nutrient agar medium (peptone, beef extract, NaCl and agar-agar) and 5mm diameter paper disks of Whatman No. 1 were used. The test compound was dissolved in DMSO in 0.1-0.4%

concentrations. The paper disks was soaked in different solutions of the compound, dried and placed in the Petri plates (9 cm diameter) previously seeded with the test organisms. The plates were incubated for 24-30 h at 27±1 ^{0}C and the inhibition zones (mm) were measured around each disk.

c- In vitro stydy: Human liver carcinoma cell line (HepG2) and two breast cancer cell lines (MCF7 and T47D) were used for *in vitro* screening experiments for the zinc complex. The cancer cells were obtained frozen in liquid nitrogen (-180^{0}C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub culturing. Cell culture cytotoxicity assays were carried out as described in literature (Shrivastava, H., *et al*., 2004). RPMI-1640 medium (sigma chemical Co, St, Louis, Mo and USA) was used for culturing and maintenance of the human tumor cell lines. Cells were seeded in 96-well microliter plates at a concentration of 5 $\times10^4$ – 10^5 cell/well in a fresh medium and left to attach to the plates for 24 h. growth inhibition of cells was calculated spectrophotometrically using a standard method with the protein-binding dye sulforhodamine B (SRB) (Sunil, D., *et al*., 2013). The optical density (OD) of each well was measured at 564 nm with an ELIZA microplate reader (Meter Tech. ∑ 960, USA). The sensitivity of the human tumor cell lines were determined by the SRB assay. The percentage of cell survival was calculated as follows:

Survival fraction = OD (treated cells) / OD (control cells)

The IC_{50} value is the concentration of thymoquinone required to produce 50% inhibition of cell growth. The results compared with a similar run of cis-platin as an antitumor compound.

d- In vivo stydy:

Materials:

- EAC cells: Cells were obtained from American Type Tissue Culture Collection, Manassas, VA, USA. Cells were maintained in *vivo* in Swiss *albino* mice by intraperitoneal (i.p.) transplantation of 2×10^6 cells/mouse after every 8 days (Ramakrishna, Y., *et al*., 1984).
- Vehicle: The zinc complex was freshly dissolved, directly before use, in a vehicle containing dimethyl sulfoxide (DMSO): H_2O, (4:1 v/v).

Methods:

Animals management:

200 Male Swiss *albino* mice (8-10 weeks of age, 27-30g body weight) were obtained from King Fahed Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were kept for one week acclimatization period under controlled condition of temperature (23-25C^0), humidity (50-55%) and light, dark cycle (12hL/12hD). Animals had free access to water and standard diet throughout the experiment.

Cytotoxicity studies and Groups:

150 mice were used in this part. Mice were randomly and equally divided into five main groups. Water and standard diet were given to mice ad libium during the whole experimental period. 5 Mice from each group were followed for determination of life span prolongation, while the rest of mice were used for determination of other biochemical parameters. The groups were include:

Group 1(control group): the mice in this group were intraperitoneally (i.p.) injected with 0.2 ml saline, 3 times/week for 14 days.

Group 2 (EAC group): the mice in this group were (i.p.) injected with $2x10^6$ Ehrlich ascites carcinoma cells/mouse and were monitored for 14 days.

Group 3 (vehicle group): the mice were ip. injected with 0.2 ml of the vehicle used to dissolve (BBIA- Zn) complex (DEMSO:H2O, 4:1 v/v)(3 times/week for 14 days).

Group4 (complex group): the mice in this group were (3 times/week for 14 days) i.p. injected with 0.2ml from 10% of the lethal dose (LD_{10}) of the complex dissolved in vehicle (DMSO: H_2O) at a final dose equivalent to 71 mg of (BBIA- Zn) complex.

Group5 (treated group): the mice in this group were inoculated i.p. with $2x10^6$ Ehrlich ascites carcinoma cells/mouse followed, after 24hr, by daily i.p. injected with 71 mg (BBIA- Zn) complex / kg body weight (o.2ml from LD_{10} of the complex) dissolved in vehicle, 3 times/week for 14 days.

Blood sample, organs Ascetic Fluid collection:

Animals were monitored for alterations in body weight, for the development of any signs of toxicity and mortality. Body weight was registered every third day till the end of the experimental period.

After the last dose, five mice from each group were left for survival study, while the rest of animals were fasted overnight, blood was withdrawn from four mice in equal amount were pooled on EDTA; resulting in five samples in each group; to obtain sufficient quantity of serum for determination of biochemical parameters.

Cytotoxicty Parameters:

i-Change in body weight:

Body weight was registered for each mouse at begging of the experiment and every 3 days till day 15. The percent of change in body weight was calculated using the following formula (Kuttan, R., *et al*., 1985):

Percentage change in weight = $(W_2 - W_1) / W_1 \times 100$

W_1 is the body weight of mouse at start of experiment and W_2 is the body weight of mouse before anatomy.

ii- Measurement of percentage increase in life span:

Animal survival time, for each group, was recorded and was expressed as mean survival time (days). Both MST and LS (%) were calculated using the equations described by Mazumder *et al.*, (1997) and Gupta *et al.*, (2000):

MST = (Day of 1st death + Day of last death) / 2

% LS = [(MST of treated group/ MST of control) - 1] × 100

iii- Ascetic fluid Collectiont:

The fluid was centrifuged to collect EAC cells, the cells were tested for viability by trypan blue using hemocytometer. The total numberof cells/ ml was calculated using the following equation:

Cell count = (No. of cells ×dilution) / Area ×thickness of liquid film

Hematological parameters

Blood was obtained from retro-orbital sinus of the mice under light ether anesthesia using heparinized micro-capillaries. Red blood cells (RBCs). White blood cells (WBCs) and hemoglobin levels were determined by using auto hematology analyzer (BC-2800Vet, Mindray, China).

Biochemical Assays:

Separated sera were used for determination of liver and kidney functions by measuring: activities ofALT, and AST activity, ALP, serum urea level, creatinine level, serum uric acid level; in addition to levels of:serum glucose, serum triglyceride, serum cholesterol, low density lipoprotein, HDL, LDH, CK, CK-MB, TAC, MDA level, SOD, CAT, GST. All the biochemical parameters were determined using specific kit for each parameter.

Statistical analysis:

Statistical analysis was performed using SPSS 22.0 for windows (SPSS Inc, USA). Descriptive statistics were shown as means and standard error of means and percentages to describe the continuous data. One-way analysis of variance (ANOVA) was performed for comparing more than two groups. Scheffe test was used as a post hoc test to compare the means of two groups to determine if they were statistically different. Eta squared and cohen's d tests were used to estimate the effect of size that describe the proportion of total variability attributable to a variable. Independent-sample t test (t) was performed for comparing means for EAC & treated groups in tumor volume (ml), viable cell count, non-viable cell count, percentage of viable cells (%) and percentage of non-viable cells (%) variables. *P* value smaller than 0.05 was considered statistically significant.

Results:

Antimicrobial activities of the BBIA- Zn complex:

The free ligand and zinc complex were screened against the E. coli as Gram–negative bacteria and S. aureus as Gram-positive bacteria, and the two fungus A. flavus and C. albicans to assess their potential activity relative to the two standard: Tetracycline antibacterial agent and Amphotericin B antifungal agent. The data showed activities of the BBIA- Zn complex against two bacteria and two fungus.

In vitro study:

The BBIA- Zn complex exhibited IC_{50} value of 5.1 µg/ml against the studied Human liver carcinoma HepG2 cell line nominated it to be considered as strong antitumor agent. The zinc complex was exhibited against two breast cancer cell lines mainly MCF7 and T47D. As expected, the complex showed activity of strong antitumor agent: IC_{50} value were 3.9 and 4.2 µg/ml for MCF7 and T47D, respectively.

In vivo study:

The dose response curve of the healthy mice injected with BBIA- Zn complex (Figure 1).

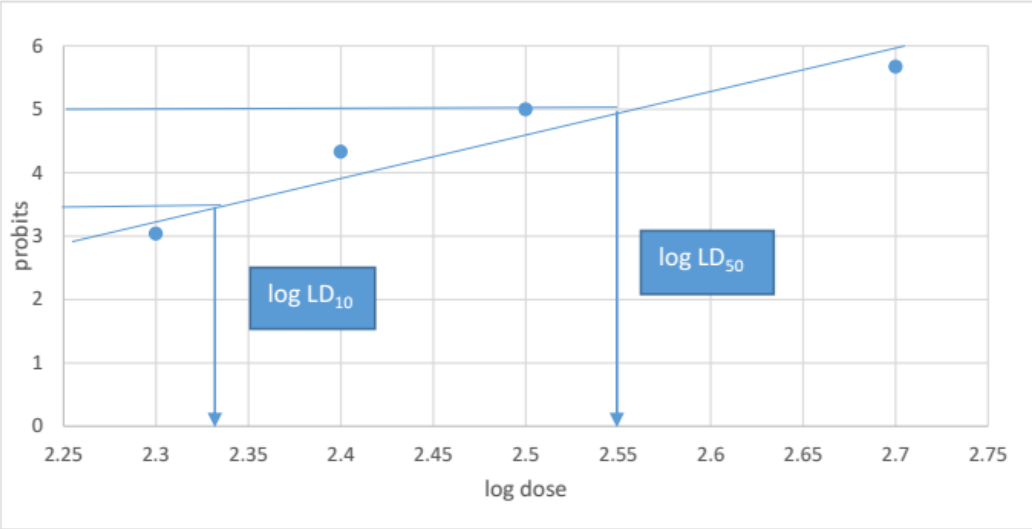

Fig. 1: The dose response curve, LD_{50} and LD_{10} values of the BBIA- Zn complex in normal mice.

Cytotoxicity study:

i-Changes in body weight:

The percent changes in body weight, in all studied groups, between day zero and day 15 are shown in Figure 2. Mice inoculated with EAC tumor cells showed a maximum gain in body weight that amount to 35%,which was significantly higher than the percent increase in normal mice.

Treatment by BBIA- Zn complex after tumor inoculation resulted in a net gain in body weight of 15%, which was not significant compared to normal mice.

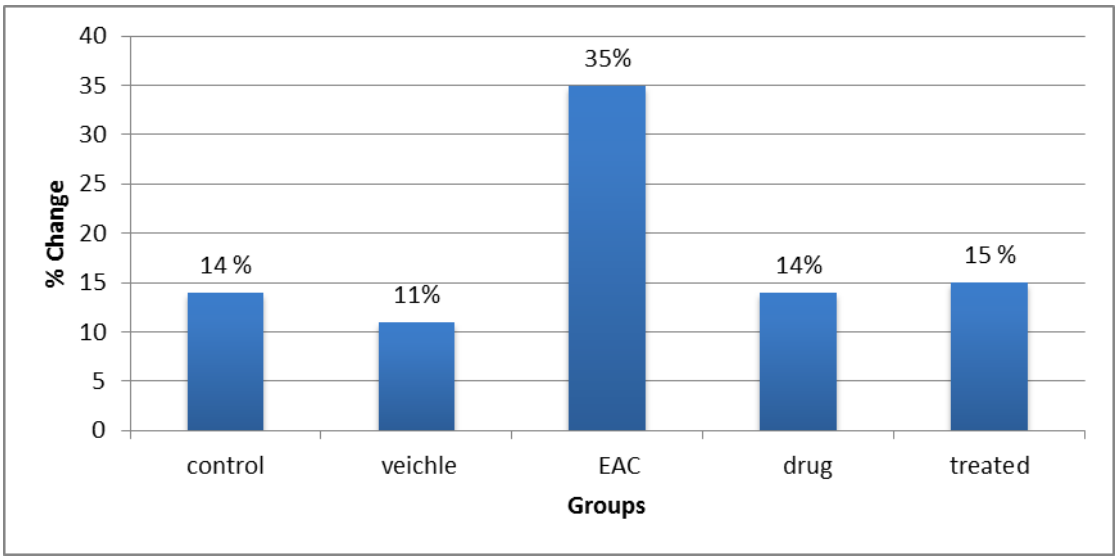

Fig. 2: The mean value of the percent change in body weight between day zero and day 15 for each group.

ii- Effect of the BBIA- Zn complex on mean survival time (MST) and percentage increase life span (%ILS):

Groups using EAC group as a control for treated group, while, normal mice group was control for zinc complex group. It is worth noting to indicate that the survival of mice in both normal and vehicle group were monitored for 120 days, no death in both groups was noted. Treatment with 71 mg zinc complex / kg after EAC inoculation improved the mean survival time of mice which in turn resulted in prolonged life span. Normal mice injected with a dose equivalent to LD_{10} dose of zinc complex showed reduction in their MST resulting in decreased life span (Table 1).

Table 1: Effect of BBIA- Zn complex on mean survival time (MST) and percent increase in life span (%ILS) in all groups:

Groups / Parameters	Control	Vehicle	EAC	BBIA -Zn	Treated
MST (days)	120	120	26.5	71	45
ILS (%)				-40.8	69.8

iii -Effect of BBIA- Zn complex on tumor volume and cell viability:

Tumor volume, viable cell count and percent of cell viability were significantly increased in tumor bearing mice group. Injection of 71 mg/kg body weight of zinc complex to EAC cells inoculated mice had significantly increased non-viable cell count and consequently decreased cell viability with inhibition of ascetic fluid accumulation (Table 2).

Table 2: Effect of BBIA- Zn complex on tumor volume and EAC cell viability:

Groups / Parameter		EAC	Treated	P-value
Tumor volume (ml)		12.963 ± 2.434	3.250 ± 0.865	0.014
Cell count	Viable (x 10^5cells/mouse)	10.338 ± 1.622	3.829± 0.421	0.040
	Non-Viable (x 10^5cells/mouse)	1.600 ± 0.282	7.357 ± 0.857	0.003
Percentage of viable cells (%)		88.275 ± 1.280	34.271 ± 1.541	0.003
Percentage of non-viable cells (%)		11.638 ± 1.277	65.643 ± 1.547	0.003

- *P* value< 0.05 is significant.
- NS: non significant, *P* value >0.05.

iv - Hematological profile:

Results indicated that inoculation of EAC cells to mice resulted in significant reduction in Hb content and RBCs count, with elevated WBCs compared to normal mice group. Significant improvement in hematological parameter, (Hb, RBCs and WBCs) was obvious by treating EAC inoculated mice with BBIA- Zn complex, reaching WBCs mean value comparable to normal (Table 3).

Table 3: Effect of zinc complex on Hb, RBCs and WBCs count in all studied groups:

Groups / Parameters	Control	Vehicle	EAC	BBIA -Zn	Treated
RBC (x10^6 cell / mm^3)	8.559 ± 0.118	8.583 ± 0.268	7.194 ± 0.134	8.017 ± 0.218	7.933 ± 0.128
*p				0.001	0.01
**p			0.01	0.001	
WBC (x10^6 cell / mm^3)	13.25 ± 0.537	8.58 ± 0.849	35.03 ± 1.684	13.83 ± 0.961	27.08 ± 1.252
*p		0.02	0.001		NS
**p		0.01		0.001	0.001
Hb (gm/dl)	14.520 ± 0.284	14.317 ± 0.443	12.731 ± 0.322	13.667 ± 0.371	14.017 ± 0.250
*p		NS	0.01	0.01	
**p		0.01			

**p*: p value with respect to normal group, ***p*: p value with respect to EAC group.
*p*value ≤ 0.05 is significant, NS: non-significant.

v - Biochemical studies:

1. Effect of BBIA- Zn complex on liver and kidney function tests:

Results revealed significant elevation in the activities of ALT, AST, ALP urea, creatinine and Uric acid and reduced total proteins, albumin and globulin was obtained after 15 days of EAC inoculation. Injection of BBIA-Zn Complex to EAC bearing mice had significantly adjusted mean values of total proteins, albumin and globulin reaching mean values comparable to healthy animals. Treatment by 71 mg BBI- Zn complex / kg B Wt had significantly restored the uric acid to normal value (Table 4).

Table 4: Effect of BBI – Zn complex on liver and kidney function in all studied groups:

Groups / Parameters	Control	Vehicle	EAC	BBIA -Zn	Treated
ALT (U/L)	31.567 ± 1.282	35.017 ± 1.132	61.928 ± 0.997	31.947 ± 0.565	34.364 ± 1.104
*p	-		0.001	0.001	0.001
**p	-		-	NS	NS

AST (U/L)	23.00 ± 0.773	36.33 ± 0.758	68.50 ± 3.175	26.90 ± 1.026	33.41 ± 1.055
*p	-	0.01	0.001	0.01	0.01
**p	-	0.001	-		
ALP (U/L)	48.68 ± 1.217	46.98 ± 1.208	92.36 ± 1.340	45.20 ± 1.900	40.95 ± 0.990
*p	-	0.001			0.01
**p	-	0.001			
Total protein (g/dl)	4.560 ± 0.027	4.733 ± 0.064	3.828 ± 0.068	4.400 ± 0.045	4.318 ± 0.026
*p	-	0.01	0.001	0.001	0.001
**p	-	0.001		0.01	0.001
Urea (mg/dl)	69.33 ± 2.773	69.83 ± 1.417	79.72 ± 1.123	64.60 ± 0.888	65.00 ± 1.421
*p	-			0.042	0.001
**p	-			0.03	0.001
Creatinine (mg/dl)	0.280 ± 0.011	0.317 ± 0.009	0.339 ± 0.012	0.300 ± 0.000	0.227 ± 0.014
*p	-				0.001
**p	-				0.001
Uric Acid (mg/dl)	0.270 ± 0.011	0.27 ± 0.009	0.38 ± 0.012	0.24 ± 0.000	0.25 ± 0.014
*p	-			0.01	
**p	-		0.01	0.001	0.001

*p: p value with respect to normal group, **p: p value with respect to EAC group.
$p$value ≤ 0.05 is significant, NS: non-significant.

2. Serum Glucose, Lipid and cardiac profile:

The current study demonstrated significant elevation in serum glucose, triglyceride, CHOL, VLDL, LDL, LDH, CK and CK-MB in untreated EAC bearing mice group. Treatment with BBIA- Zn-Complex had almost significantly restored to normal value (Table 5).

Table 5: Effect of BBIA- Zn-Complex on serum Glucose, Lipid and cardiac profile for the studied groups:

Groups / Parameters	Control	Vehicle	EAC	BBIA -Zn	Treated
Glucose (mg/dl)	91.80 ± 4.334	98.67 ± 0.536	60.00 ± 2.506	99.20 ± 2.277	75.45± 7.109
*p	-	0.001	0.001	0.001	0.001
**p	-	0.001	-	0.001	0.001
Triglycerides (mg/dl)	24.00 ± 0.460	43.17 ± 0.984	68.50 ± 3.807	26.30 ± 1.064	21.68 ± 1.174
*p	-	0.01	0.001	0.001	0.001
**p	-	0.001	-	0.001	0.001
CHOL (mg/dl)	37.30 ± 3.724	43.42 ± 2.695	52.17 ± 3.233	26.40 ± 3.101	29.73 ± 8.901
*p	-	0.02	0.001	-	NS
**p	-	0.001	-	0.001	0.001
VLDL (mg/dl)	20.40 ± 0.496	21.17 ± 0.923	34.17 ± 1.027	21.20 ± 0.571	21.36 ± 0.742
*p	-	0.001	0.001	NS	0.01
**p	-	NS	-	0.001	0.001
LDL (mg/dl)	36.90 ± 0.855	30.67 ± 0.824	65.25 ± 1.560	29.50 ± 0.965	25.50 ± 0.634
*p	-	0.001	0.001	0.001	0.001
**p	-	0.002	-	NS	NS
HDL (mg/dl)	41.80 ± 3.368	46.25 ± 2.198	18.00 ± 1.832	44.80 ± 1.082	49.77 ± 0.868
*p	-	0.001	0.01	0.001	0.001
**p	-	-	0.001	NS	NS
LDH(U/L)	24.20 ± 3.732	30.50 ± 11.565	68.50 ± 14.63	17.60 ± 5.465	54.00 ± 2.703
*p	-	0.001	0.001	0.001	0.001
**p	-	0.001	-	0.001	0.001
CK(total) (U/L)	25.50 ± 2.717	33.08 ± 3.032	63.83 ± 4.886	34.40 ± 4.666	32.73 ± 7.349
*p	-	0.002	0.001	0.001	0.001
**p	-	0.001	-	NS	NS
CK-MB (U/L)	58.780 ± 1.808	56.750 ± 1.653	83.400 ± 1.22	53.420 ± 1.53	64.555 ± 1.97
*p	-	0.001	0.001	0.001	0.001
**p	-	NS	-	0.001	0.001

*p: p value with respect to normal group, **p: p value with respect to EAC group.
p value ≤ 0.05 is significant, NS: non-significant.

3. Effect of BBIA- Zn complex on antioxidant activities:

Results demonstrated significant reduction in total antioxidant capacity, SOD, CAT, with elevated MAD in animals inoculated with EAC compared to normal mice. EAC inoculated mice treated with BBIA- Zn complex had a pronounced improvement in the antioxidant status compared to non treated EAC group. (Table 6).

Table 6: Effect of BBIA- Zn complex on antioxidant activities in all studied groups:

Groups Parameters	Control	Vehicle	EAC	BBIA -Zn	Treated
TAC (mmole/L) *p **p	1.349 ± 0.058 - -	1.201 ± 0.105 0.01 0.001	0.868 ± 0.055 0.001 -	1.275 ± 0.067 0.001 0.001	1.525 ± 0.054 0.001 0.001
MDA (nmole/L) *p **p	23.40 ± 0.703 - -	33.67 ± 0.657 0.001 -	68.50 ± 1.104 0.01 0.001	33.60 ± 0.892 0.001 NS	28.09 ± 0.521 0.001 NS
SOD (U/mL) *p **p	158.17 ± 5.94 - -	164.30 ± 8.95 NS 0.01	100.46 ± 0.59 0.01 -	169.21 ± 4.11 0.01 0.001	160.24 ± 9.25 NS 0.001
CAT (U/mL) *p **p	0.446 ± 0.023 - -	0.516 ± 0.027 0.01 0.001	0.143 ± 0.019 0.001 -	0.575 ± 0.003 0.001 0.001	0.506 ± 0.021 0.001 0.001
GST (U/mL) *p **p	14.600± 0.139 - -	14.767 ± 0.18 0.02 0.001	9.837 ± 0.230 0.001 -	15.120 ± 0.14 0.01 0.001	14.436 ± 0.33 NS 0.001

*p: p value with respect to normal group, **p: p value with respect to EAC group.
p value ≤ 0.05 is significant, NS: non-significant.

Discussion:

The major focus of research in chemotherapy for cancer includes the identification, characterization and development of new and safe cancer chemo- preventive agents. In the current study, the anticancer activity of a novel synthetic zinc complex of Bis (4-bromobenzaldehyde-4-iminacetophenone) Diaquozinc (II) Nitrate (BBIA- Zn complex) was estimated. The *in vitro* cytotoxicity of the complex against breast (MCF7 & T47D) and human liver (HepG2) carcinoma cell lines (Ramadan, M., *et al*., 2015). The present study was carried out to evaluate the antitumor effect and antioxidant status of BBIA- Zn complex in EAC-bearing mice. The treated animals significantly inhibited the tumor volume, packed cell volume, viable tumor cell count, increased the mean survival time, peritoneal cell count. They also restored the hematological parameters to more or less normal levels. They decreased the hepatic lipid peroxidation and increased the antioxidant enzyme SOD and CAT as well as the GST level. The mean survival time (MST) of untreated EAC bearing mice was 26.5 days. Because of rapid EAC cell division during the proliferating phase, ascites fluid accumulated, resulting in host animal death due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor itself (Altun, S., 1996). Treatment with BBIA- Zn complex resulted in increased MST, reaching 45 days, with percentage increase in life span equivalent to ~65 %. This result might point out to effectiveness of the complex as anticancer agent. One of the reliable criterion for judging the value of anticancer drug is the prolongation of animal life span (Clarkson, D. and J.H. Burchneal, 1998), where an enhancement of life span up to 25% or more was considered as an effective anticancer (Naveena, Bharath B.k. and Selvasubramanian, 2011).

Inoculation of EAC cells to mice, in the current research, resulted in significant increase in body weight after 15 days of inoculation (35.5 %) compared to normal mice, which could be attributed to tumor burden. The Ehrlich tumor cells are aggressive rapidly growing cells (Segura, J.A., *et al*., 2000) which can grow in different mice strains (Ahmed, H., *et al*., 1988). The implantation of EAC cells induces local inflammatory reaction with increasing vascular permeability resulting in edema, cellular migration and a progressive ascetic fluid formation (Fecchio, D., *et al*., 1990). The ascetic fluid constitutes nutritional source for the cells, therefore, it is essential for tumor cell growth (Shimizu, M., *et al*., 2004). Treating EAC group with BBIA- Zn complex had prevented the accumulation of ascetic fluid resulting in reduction in weight gain (14.8%); almost comparable to normal mice group; at the end of experiment.

In cancer chemotherapy, the major problems encountered are mylo-supperession and anemia (Hogland, H.C., 2010). In the present study, tumor induction had significantly decreased RBC and hemoglobin contents and increased WBC count. The anemia encountered in tumor bearing mice is mainly due to a reduction in RBC or hemoglobin percentage which may be due to iron deficiency or due to hemolytic or myelopathic conditions. Recovery of the hemoglobin content, RBC and WBC count observed by treatment indicated the protective action of zinc complex on the heamopoietic process. The present study indicated that EAC cells inoculation had significantly induced hepatotoxicity in mice as manifested by elevated ALT, AST and ALP activities and reduced total proteins, indicating hepatocellular damage by EAC cells. Significantly elevated levels of alanine

transaminase (ALT) is characteristics of viral hepatitis, diabetes, liver damage, bile duct problems and liver cancer, so ALT is commonly used as a way of screening for liver problems (Paul, T and M. Giboney, 2002). AST is similar to ALT in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle. ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs (Karmen, A., *et al*., 2005). ALP Levels are also elevated in people with untreated liver disease (Abou Zaid, O.A.R., *et al*., 2011). Damaged hepatocytes of EAC bearing mice might be due to cytotoxic agent itself or due to its toxic metabolites (Pizzuti, G.P. and G.C. Salvatori, 2012). In a previous studies elevated AST and ALP was attributed to increased consumption of amino acids for building proteins of rapidly dividing tumor cells resulting in disturbing the activities of liver enzymes (Lange, P.H., *et al*., 2013). In the current work, the damaging effect observed by EAC inoculation was improved which can be explained by prevention of tumor growth after administration of antitumor BBIA- Zn complex at a dose equivalent to LD_{10} value/3 times a week for two weeks

In the current study, EAC bearing mice had significantly elevated serum urea. It is established that EAC proliferation in mice causes kidney damage and elevated blood urea. Increased urea synthesis and hence excretion in EAC inoculated mice was attributed to enhanced activities of the enzymes involved in urea cycle (Greenan, N.S., *et al*., 2005). When there is kidney damage or kidney disease, and the kidneys are not able to filter waste efficiently, there will likely be a rise in creatinine levels in the blood (Persky, A.M. and E.S. Rawson, 2007). Biochemical parameters of EAC bearing mice showed a protective effect of BBIA- Zn complex against kidney damage induced by EAC inoculation. On the other hand, remarkable increase in serum uric acid mean value was obtained in EAC bearing mice group. Treatment with BBIA-Zn had significantly restored uric acid to normal values. The elevated serum uric acid in EAC bearing animals could be due to the malignant process itself, resulting from the increased nucleic acid turnover in the rapidly proliferating diseased tissue (Sevanian, A., *et al*., 1991).

The current study demonstrated that EAC inoculation to mice had resulted in a significant decrease in blood glucose compared to normal mice. Most studies had observed lower serum glucose in tumor bearing mice (Naveena, Bharath B.k. and Selvasubramanian, 2011). One reason for hypoglycemia could be an augmented consumption of glucose by the cells of the tumor (Nakamura, W. and S. Hosoda, 1968). Anaerobic glycolysis is an important step in the growth of tumour and Zn-co by exerting its effect through inhibiting Hexokinase or by lowering the levels of Fructose-2,6-bisphosphate and reduced allosteric effect on PFK or by an unidentified mechanism inhibit it.As indicated in the current study, treatment had resulted in increased serum glucose, and it was restored to approximately similar values as in normal control (group I).

In the current study, lipids were deranged in EAC bearing mice, where significantly elevated in TAG, cholesterol and VLDL levels were noted. There is an increasing evidence that cancer cells show specific alterations in different aspects of lipid metabolism. Changes in lipid metabolism can affect numerous cellular processes, including cell growth, proliferation, differentiation and motility (Beckers, A., *et al*., 2007). Several mechanisms have been proposed by different authors to explore the hypertriglyceridemia and elevated VLDL associated with EAC bearing mice. Lyon *et al*., (1982) attributed the elevated TAG to defective removal of VLDL-TAG. Others (Kannan, R and N. Baker, 1977) reported that fasting lowers plasma TAG in mice, but chronically lowered insulin levels and other hormonal changes that followed reduced food intake might cause inhibition of VLDL-TAG removal from the circulation. The high degree of lipemia in tumor bearing animals could be also due to mobilization of body fat for energy production (Pratt, A.W., 1957). Tumor load promotes the breakdown of lipids in the adipose tissue of cachexic patients. Tumor cells can use circulating free fatty-acids as an energy supply, for membrane biosynthesis or for signaling processes. Glycerol produced by the breakdown of triacylglyceridescan be used for gluconeogenesis in the liver (Hager, M.H., *et al*., 2006), which enters the peritoneal cavity. TAG and cholesterol contained in VLDL are taken up and utilized by Ehrlich cells, suggesting that one function of VLDL is to transport lipids from host tissue to the growing tumors (Lyon, I., *et al*., 1982). In this study, although treatment with BBIA-Co complex had ameliorated some of the abnormalities induced by EAC inoculation, however the complex was unable to improve the changes in lipid profile. EAC bearing mice had reduced of high density lipoprotein. Researchers at the Molecular Cardiology Research Institute at Tufts Medical Center in Boston conducted an analysis of 24 trials that investigated the pros and cons of cholesterol treatment interventions, primarily statin therapy. The research team looked at high-density lipoprotein (HDL) cholesterol, the "good" cholesterol that is protective against heart disease, and the incidence of cancer (Chapman, M.J., *et al*., 2009). HDL is responsible for transporting cholesterol to the liver and keeping cholesterol moving throughout the body. Higher HDL is associated with a twofold to threefold reduced risk for heart disease. The biological mechanisms in which HDL might also protect from cancer is unclear, the researchers say. Treatment of EAC bearing mice with BBIA- Zn complex reduced to a great extent the deleterious effect of tumor on lipid components (Hirano, T., *et al*., 2008).

EAC bearing mice had increased lactate dehydrogenase. LDH is involved in tumor initiation and metabolism. Cancer cells rely on increased glycolysis resulting in increased lactate production instead of aerobic

respiration in the mitochondria, even under oxygen-sufficient conditions a process known as the Warburg effect (2003). For this reason, LDHA and the possibility of inhibiting its activity has been identified as a promising target in cancer treatments focused on preventing carcinogenic cells from proliferating. The present investigation indicated significantly reduced relative heart weight in mice bearing tumor. In addition, tumor resulted in significant cardiac functional deteriorations as characterized by increased cardiac enzymes (LDH, CK and CK-MB). CK-MB is present in the myocardium that leaks during massive myocardial damage resulting from disintegration of the contractile apparatus and increased sarcoplasmic permeability. The BBIA- Zn complex may be due to the protective action of the complex to cardiac membrane against free radicals and decreased lipid peroxidation level that prevented the release of cardiac enzymes.

Lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to cancer and other genetic diseases linked to lifestyle and dietary factors (Stewart, A.J. and N. Pellegrini, 2005). Total antioxidant capacity, SOD and CAT activities were significantly reduced, while levels of MDA showed higher mean value in EAC animals group relative to control. In this study, both SOD and CAT activities were appreciably elevated while MDA was significantly reduced by treatment, suggesting that, BBIA- Zn complex can restore the antioxidants statues of the treated mice. Elevated glutathione-s-transferase (GST) was clear by i.p. injection of the complex to normal mice. GST is detoxification enzyme that is involved in direct detoxification as well as acting as an inhibitor of the Mitogen-activated protein (MAP) kinase pathway, therefore protects cellular macromolecules from attack by electrophiles. The link between GST and cancer is most obvious in the overexpression of GST in many cancers, but it is also supported by the fact that the transformed phenotype of tumor cells is associated with aberrantly regulated kinase signaling pathways and cellular addiction to overexpressed proteins. That most anti-cancer drugs are poor substrates for GST indicates that the role of elevated GST in many tumor cell lines is not to detoxify the compounds, but must have another purpose; this hypothesis is also given credence by the common finding of GST overexpression in tumor cell lines that are not drug resistant (Tew, K.D., *et al*., 2011).

It is very difficult to suggest the possible mechanism of these compounds for anticancer effects. The compounds had shown promising cytotoxic activity when screened using the in vitro method and at the same time were shown to have good activity when tested using the Ehrlich ascites carcinoma model. The activity of the metal complex may be retained to the lipophilic nature of the complex which arose from the chelation. It was also noted that the toxicity of the metal complex increase on increasing the metal ion concentration. This elevation is probably due to faster diffusion of the chelates as a whole through the cell membrane. The chelated metal may block the enzymatic activity of the cell or it may catalyze the toxic reaction among cellular constituents. Guo *et al.,* (2016) studied that ruthenium(II) complex can inhibit the proliferation via blocking cell cycle progression and inducing reactive oxygen species-dependent and mitochondria-mediated apoptosis. The data showed by Ramadan *et al*., (2015) that the free ligand has the capacity of inhibiting the metabolic growth of the investigated bacteria and the fungus to different extents, which may indicate broad-spectrum properties. The activity of zinc complex may be arising from the presence of the C=N and C=O moieties. The mode of action may involve the formation of hydrogen bonding between these functional groups and the active centers of the cell constituents, resulting in inference with the normal cell process. Although the exact cellular targets responsible for their antitumor properties are unclear, they are all directly cytotoxic to tumor cells and appear to have antimitochondrial activity. The mechanism of their antitumor properties is less clear but could possibly involve interference with oxidative phosphorylation, induction of mitochondrial-dependent apoptosis or other mechanisms (Mark, M., 2002). The compounds which exhibit IC_{50} in the range of 10-25 µg/ml are considered weak anticancer drugs. On the other hand, the compounds show IC_{50} values in the range between 5 and 10 µg/ml are moderate antitumor agents, while those exhibit IC_{50} activity below 5 µg/ml are considered strong antitumor agents (Cutillas, N., *et al*., 2013).

The BBIA- Zn complex exhibited IC_{50} value of 5.1 µg/ml against the studied HepG2 cell line nominated it to be considered as strong antitumor agent. The BBIA- Zn complex was exhibited against two breast cancer cell line mainly MCF7 and T47D. As expected, the complex showed activity of strong antitumor agent: IC_{50} value were 3.9 and 4.2 µg/ml for MCF7 and T47D, respectively. On comparing the IC_{50} values of the BBIA- Zn complex with the standard cis-platin, it can be noticed that the BBIA- Zn complex has better activity against the studied cell lines than the cis-platin reagent (Shier, W.T., 2007).

The expected mechanism of action of the tested aqua complexes at the molecular level is covalent cross-linking of DNA nitrogen nucleophiles (Cutillas, N., *et al*., 2013). The complex had significantly protected the vital organs: spleen, heart, kidney and lungs against damage induced by EAC cell inoculation. Treatment had restored the antioxidant status of tumor bearing mice toward more or less normal.

Conclusion: The current investigation is considered as a first study. The overall finding of this study confirms the therapeutic effect of the zinc complex against EAC cells subcutaneously inoculated to mice, the complex showed activity of strong antitumor agent. Improvement of the antioxidant status might be responsible for the potent antitumor activity of the drug. Further studies are required to explore the mode of action of zinc complex as a cytotoxic agent.

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REFERANCES

Abou Zaid, O.A.R., M.R.R. Hassanein, Y.A. EL-Senosi and M.F. EL-Shiekha, 2011. Biochemical Effect of some Antioxidant on Metabolic Changes in Experimentally Induced Tumor in Female Mice. Benha Veterinary Med. J, pp: 52-60.

Ahmed, H., B.P. Chatterjee and A.K. Debnath, 1988. Interaction and *in vivo* Growth Inhibition of Ehrlich Ascites Tumor Cells by Jacalin. J Biosci, 13: 419-424.

Altun, S., 1996. Normal, ümöralve Rejeneratif Büyümeler Arasındaki Kinetik İlişkiler. Tradit. J. Biol., 20: 153-173.

Amin, M., M.S. Iqbal, R.W. Hughes, S.A. Khan, P.A. Reynolds, V.I. Enne, Sajjad-ur-Rahman, Mirza AS 2009. Mechanochemical synthesis and in vitro anti Helicobacter pylori and urease inhibitory activities of novel zinc (II) famotidine complex. Biochem, 25: 383-90.

Andrews, M. and C. Gallagher-Allred, 2009. The role of zinc in wound healing. World J. Sci., 12: 137-138.

Beckers, A., S. Organe, L. Timmermans, K. Scheys, A. Peeters, K. Brusselmans, G. Verhoeven and J.V. Swinnen, 2007. Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. Cancer Res., 67: 8180-8187.

Brabes, V. and O. Novakova, 2006. DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity. Drug Resist Update, 9: 111-122.

Chapman, M.J., G. Assmann, J.C. Fruchart, J. Shepherd and C. Sirtori, 2009. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid--a position paper developed by the European Consensus Panel on HDL-C. Current Med Res., 20: 1253-1268.

Clarkson, D. and J.H. Burchneal, 1998. Preliminary screening of antineoplastic drugs. Progclin cancer, 1: 625-629.

Cutillas, N., G.S. Yellol, C. Haro, C. Vicente and V. Rodriguez, 2013. Metal complexes and their antimicrobial activity. Chem Rev., 257: 2784-2797.

Devi, P.U., F.E. Solomon and A.C. Sharada, 2005. In vivo tumor inhibitory and radiosensitizing effects of an Indian medicinal plant, Plumbagorosea on experimental mouse tumors. Indian J ExpBiol, 32: 523-528.

Eckhardt, A.E., B.N. Malone and I.G. Goldstein, 2006. Inhibition of Ehrlich ascites tumor cell growth by Griffoniasimplicifolia I lectin in vivo. Cancer Res., 42: 2977-2979.

Failli, P., D. Bani, A. Bencini, M. Cantore, L. Di Cesare Mannelli, C. Ghelardini, C. Giorgi, M. Innocenti, F. Rugi, A. Spepi, R. Udisti, B. Valtancoli, 2009. A novel manganese complex effective as superoxide anion scavenger and therapeutic agent against cell and tissue oxidative injury. J Med Chem, 52: 73-83.

Fecchio, D., P. Sirois, M. Russo and S. Jancar, 1990. Studies on Inflammatory Response Induced by Ehrlich Tumor in Mice Peritoneal Cavity. Inflammation, 14: 125-131.

Garen, A. and C. Levinthal, 2001. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of E. coli. I. Purification and characterization of alkaline phosphatase. BiochimBiophysActa, 38: 470-83.

Göschl, S., E. Schreiber-Brynzak, V. Pichler, K. Cseh, P. Heffeter, U. Jungwirth, M.A. Jakupec, W. Berger, B.K. Keppler, 2017. Comparative studies of oxaliplatin-based platinum(iv) complexes in different in vitro and in vivo tumor models.Metallomics, 22; 9(3): 309-322.

Greenan, N.S., R.L. Mulvaney and G.K. Sims, 2005. A microscale method for colorimetric determination of urea in soil extracts". Communications in Soil Science and Plant Analysis. Eur J Biochem, 26: 2519-2529.

Guo, L., L. Qiu, H. Yang, L. Zhang, M. Zou and J. Lin, 2016. Insights into anticancer activity and mechanism of action of a ruthenium(II) complex in human esophageal squamous carcinoma EC109 cells.Eur J Pharmacol., 6: 78-88.

Gupta, M., U.K. Mazumder, N. Rath and D.K. Mukhopadhyay, 2000. Antitumor Activity of Methanolic Extract of Cassia Fistula L. Seed against Ehrlich Ascites Carcinoma. J. Ethnopharmacol., 72: 151-156.

Hager, M.H., K.R. Solomon and M.R. Freeman, 2006. The role of cholesterol in prostate cancer. CurrOpinClinNutrMetab Care, 9: 379-385.

Hirano, T., K. Nohtomi, S. Koba, A. Muroi and Y. Ito, 2008. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. J Lipid Res., 49: 11-30.

Hogland, H.C., 2010. Hematological complications of cancer chemotherapy. SemOncol, 9: 95-102.

Jamieson, E.R. and S.J. Lippard, 2009. Structure, Recognition and processing of cisplatin DNA adducts. Chem Rev., 99: 2467-2498.

Kannan, R and N. Baker, 1977. Hypertriglyceridemia in Ehrlich Ascites Carcinomatous Mice: Tumor and Mouse Strain Differences. Lipids, 72: 153-158.

Karmen, A., F. Wroblewski and J.S. Ladue, 2005. Transaminase activity in human blood. J clin invest, 34: 126-131.

Kirschner, S., Y-K. Wei, D. Francis and J. Bergman, 2000. Anticancer and potential antiviral activity of complex inorganic compounds. J Med Chem, 9: 369-372.

Kushi, L.H., C. Doyle, M. McCullough, C.L. Rock, W. Demark-Wahnefried, E.V. Bandera, S. Gapstur, A.V. Patel, K. Andrews, T. Gansler, 2012. American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin, 62: 30-67.

Kuttan, R., P. Bhanumathy, K. Nirmala and M.C. George, 1985. Potential anticancer activity of turmeric (Curcumal longa). Cancer Lett, 29: 197-202.

Lange, P.H., J.L. Millan, T. Stigbrand, R.L. Vessella, E. Ruoslahti and W.H. Fishman, 2013. Placental alkaline phosphatase as a tumor marker for seminoma. Cancer Res., 42: 244-247.

Lettre, R., N. Paweletz, D. Werner and C. Granzow, 1992. Sublines of the Ehrlich-Lettre Mouse Ascites Tumor. New Tool for Experimental Cell Research, Naturwissenschaften., 59: 59-63.

Loewenthal, H and G. Jahn, 1932. ÜbertragungSuersucheMitCarcinomatöserMause-Asciteslussigleit Und İhrVerhaltenGegenPhysikalische Und ChemischeEinwirkungen. Z. Krebsforsch, 37: 439-447.

Lyon, I., R. Kannan, M. Ookhtens and N. Baker, 1982. Turnover and Transport of Plasma Very-Low-Density Lipoprotein Triglycerides in Mice Bearing Ehrlich Ascites Carcinoma. Cancer Res., 42: 132-138.

Mark, M., 2002. Mechanisms of cytotoxicity and antitumor activity of gold(I) phosphine complexes: The possible role of mitochondria. CoordChem Rev., 2: 43-68.

Mazumder, U.K., M. Gupta, S. Maiti and M. Mukherjee, 1997. Antitumor Activity of Hygrophilaspinosa on Ehrlich Ascites Carcinoma and Sarcoma-180 induced Mice. Indian J. Exp. Biol., 35: 473-477.

Nakamura, W. and S. Hosoda, 1968. The Absence of Glucose in Ehrlich Ascites Tumor Cells and Fluid. Biochim. Biophys. Acta, 158: 212-218.

Naveena, Bharath B.k. and Selvasubramanian, 2011. Antitumor Activity of *Alo Vera* against EAC in Swiss Albino Mice. Int J of Pharma and bio Sci., 2: 6275-6299.

Paul, T and M. Giboney, 2002. Mildly Elevated Liver Transaminase Levels in the Asymptomatic Patient. Pharm Ther, 33: 271-303.

Persky, A.M. and E.S. Rawson, 2007. Safety of creatine supplementation. Sub-cell biochem, 46: 275-89.

Pizzuti, G.P. and G.C. Salvatori, 2012. Some blood parameters of water buffalo in different physiological conditions. Boll Soc Ital BiolSper, 69: 649-654.

Pratt, A.W., 1957. Observations on the Respiratory Metabolism of Osberne -Mendel Rats Bearing Walker 256 Tumor Transplants. Proe Am Assoc Cancer Res., 2: 240.

Rafique, S., M. Idrees, A. Nasim, H. Akbar and A. Athar, 2010. Transition metal complexes as potential therapeutic agents. MolBiol Rev., 5: 38-45.

Ramadan, M., Ramadan, Ahmad K. Abu Al-Nasr, Amani F.H. Noureldeen, 2015. Synthesis, spectroscopic, antimicrobial activities and antitumor of a new monodentate V-shaped Schiff base and its transition metal complexes. SpectActaMolBiomol., 132: 417-422.

Ramakrishna, Y., A.L. Manohar, P. Mamata and K.G. Shreekant, 1984. Plants and Novel Antitumor Agents: A review. Indian Drugs, 21: 173-185.

Scozzafava, A., L. Menabuoni, F. Mincione, G. Mincione and C.T. Supuran, 2001. Carbonic anhydrase inhibitors: synthesis of sulfonamides incorporating dtpa tails and of their zinc complexes with powerful topical antiglaucoma properties. Bioorg Med Chem Letters, 11: 575-582.

Segura, J.A., M.A. Ruiz-Bellido, M. Arenas, C. Lobo, J. Marquez and F.J. Alonso, 2000. Ehrlich Ascites Tumor Cells Expressing Anti-Sense Glutaminase RNA Lose Their Capacity to Evade the Mouse İmmune System. Int. J. Cancer, 91: 379-384.

Sevanian, A., K.J. Davis and P. Hochstein, 1991. Serum Urate as an Antioxidant for Ascorbic Acid. Am J ClinNutr, 54: 1129S-1134S.

Shier, W.T., 2007. Mammalian cell culture on day: A lap manual of low cost methods. University Philippines. Los banos, 2: 19-91.

Shimizu, M., C. Azuma, T. Taniguchi and T. Murayama, 2004. Expression of Cytosolic Phospholipase A2 alpha in Murine C12 cells, a Variant of L929 Cells, induces Arachidonic Acid Release in Response to PhorbolMyristate Acetate and Ca+2Ionophores, but not to Tumor Necrosis Factor-alpha. J Pharm Sci., 96: 324-332.

Shirin, C.Z. and R.N. Mukherj, 2010. Synthesis, spectra and electrochemistry of ruthenium(III) complexes with cage-like Schiff-base ligands. Drug Resist Update, 11: 2625-2630.

Shrivastava, H., T. Ravikumar, N. Shanmugasundaram and Babo, 2004. Cytotoxicity Studies of Chromium (III) Complex on Human Dermal Fibroblasts. Free radical Bio Med., 38: 58-69.

Siems, W., 1993. Changes in the Nucleotide Metabolism of Ehrlich Ascites Tumor Cells during their growth In vivo. Cell MolBiol., 35: 255-262.

Stewart, A.J. and N. Pellegrini, 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. NutrMetabCardiovasc Dis., 15: 316-328.

Sunil, D., A.M. Isloor, P. Shetty, P.G. Nayak and K.S.R. Pai, 2013. *In vivo* Anticancer and Histopathology Studies of Schiff Bases on Ehrlich Ascitic Carcinoma Cells. Arabian J of Chem, (6): 25-33.

Tew, K.D., Y. Manevich, C. Grek, Y. Xiong, J. Uys and D.M. Townsend, 2011. The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer. Free RadicBiol Med., 51: 299-313.

Vallee, B.L and D.S. Auld, 2008. Zinc coordination, function and structure of zinc enzymes and other proteins. Biochem, 29: 5647-5659.

Vallee, B.L. and K.H. Falchuk, 2007. The biochemical basis of zinc physiology. J med Chem, 73: 79-118.

Warburg, O., 2003. On the origin of cancer cells. Science, 123: 309-14.