Antitumor and Antioxidant Activities of a Novel Platinum(II)-PyrazineCarboxamide Complex Against Ehrlich Ascites Carcinoma Induced in Mice

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ABSTRACT

The present study was motivated to evaluate the anticancer potential of platinum(II)-pyrazinecarboxamide-bipyridine complex (PCABpy-Pt) against Ehrlich ascites carcinoma (EAC) cells induced in mice. 150 mice were divided equally to five groups: control healthy mice, EAC bearing mice (mice were ip. inoculated with 2x106 EAC cells/mouse), vehicle group (mice were ip. injected with 0.2 ml of DMSO:H2O, 4:1 v/v, 3 times/week, for 2 weeks), platinum complex group (mice were ip. injected with 80 mg/Kg freshly dissolved PCABpy-Pt complex in a vehicle, 3 times/week, for 2 weeks) and treated EAC-inoculated mice group (mice were inoculated with EAC followed by treatment after 24 h with the same dose of Pt drug as in Pt complex group). Results indicated that treatment with the drug prevents the accumulation of ascetic fluid and consequently decreased the cell viability with increasing non-viable cell count, which in turn resulted in 58 % increased life span of tumor bearing mice. Hematological profile reverted to normal values by treatment. All the biochemical parameters of EAC bearing mice including liver, kidney, lipid profile and cardiac enzymes were improved by treatment reaching almost values comparable to those in healthy animals. The complex had significantly enhanced the antioxidant activity of the treated mice. Significant improvement in histopathological examinations of vital organs including heart, liver, kidney, lung and spleen were noted by treatment. No significant variations were detected in all biochemical parameters by injecting the drug to healthy animals. The study concluded that the complex proved to have a potential role as an anticancer agent probably through its antioxidant properties.

Key Words: complexes; Ehrlich carcinoma cells; Biological activity; Antioxidant studies; In vivo cytotoxicity; Histopathological studies

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INTRODUCTION

Cancer is one of the major cause of death in the world. The number of global cancer death is projected to increase 45% from 2007 to 2030 influenced in part by an increasing and aging global population. The estimated rise considers expected slight declines in death rates for some cancers in high resource countries. New cases of cancer in the same

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period are estimated to jump from 11.3 million in 2007 to 15.5 million in 2030 [1]. Current treatment of cancer is limited to surgery, radiotherapy and the use of cytotoxic agents (despite their well-known side effects and problems associated with the development of resistance). For most forms of disseminated cancer, however, no curative therapy is available, and the discovery and development of novel active chemotherapeutic agents is largely needed [2].

Transition metal-based compounds constitute a discrete class of chemotherapeutics, which are widely used in the clinic as antitumor and antiviral agents. Examples of established antitumor metallodrugs, routinely used in clinic, are *cis*-platin [cis-diamminedichloro-platinum(II)] and its analogues [3,4]. However, drug resistance and side effects have limited their clinical utility. These limitations have prompted a search for more effective and less toxic metal-based antitumor agents [5,6,7].For these reasons, vast efforts are committed to develop novel platinum-based complexes which might overcome the shortcomings and sever side effects of *cis*-platin. Therefore, researchers since then do more efforts to discover other platinum-based derivatives with higher antitumor activity and display a more tolerable toxicological profile and overcome resistance in many tumor types.

Interest in transition metal complexes of heterocyclic compounds containing nitrogen (as a donor atom) such as pyridines, bi- and polypyridines, 2-(2'-pyridyl) benzimidazole, 2-pyrazinecarboxylic acid, 2-pyrazine carboxamide and 2 aminobenzimidazole have a vital role in biology [8,9]. Recently, Ramadan and Al-Raddady [10] preliminary investigated a novel platinum complex (platinum(II)-pyrazinecarboxamide-bipyridine, PCABPY-Pt) against two breast cell lines (MCF7 and T47D) and human liver carcinoma cell line (HepG2). The IC_{50} of the reported platinumcomplex was found to be: 3.3µg/ml (5.3µM) for MCF7, 3.9µg/ml (5.7µM) for T47D and 3.15µg/ml (5.0µM) for HepG2. Results of this study indicated strong and promising anticancer activity of the complex relative to that of the more established *cis*-platin drug.

Experimental tumors have great importance for the purposes of modeling and Ehrlich ascites carcinoma (EAC) is one of the commonest. It appeared firstly as a spontaneous breast cancer in a female mouse and then Ehrlich and Apolant in 1905 [11] used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse [12]. EAC is referred to as an undifferentiated carcinoma, and has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and does not have tumor specific transplantation antigen [13]. These entire make EAC an efficient model of experimental tumor induced in animals. The present study was carried out to investigate the *in vivo* antitumor activity as well as anti-oxidative stress of PCABpy-Pt complex against EAC cells induced in male *albino* mice.

MATERIALS AND METHODS

Preparation of the platinum complex (PCABpy-Pt)

The complex under investigation was prepared according to the method described previously [10].

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 (ip.) transplantation of 2 $x10^6$ cells/mouse after 8 days [14]. EAC cells were used for cytotoxicity study (2 $x10^6$ cells/mouse in 0.2 ml).

Animals management and groups

200 Male Swiss albino mice (8-10 weeks of age, 30-33 g body weight) were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were kept for one week acclimatization period under controlled conditions of temperature (23-25 C^o), humidity (50-55%) and light, dark cycle (12 h L / 12h D). Animals had free access to water and standard diet throughout the experiment. Mice were equally divided into five main groups as follows: Normal group (mice were ip. injected with 0.2 ml saline, 3 times/week for 2 weeks), EAC group (mice were ip. inoculated with $2x10^6$ Ehrlich ascites carcinoma cells/mouse and were monitored for 14 days), Vehicle group (healthy mice were injected with 0.2 ml of DMSO: H_2O, 4:1, 3 times/week for 2 weeks. PCABpy-Pt group (animals in this group were ip. injected with LD_{10} of PCABpy-Pt complex; 80 mg of PCABpy-Pt/Kg body weight freshly dissolved in vehicle; 3 times/week for 2 weeks and treated group (animals were i.p. inoculated with EAC cells; $2x10^6$ cells/mouse; followed after 24 h, by ip. injection of LD_{10} of PCABpy-Pt complex dissolved in vehicle, 3 times/week for 2 weeks.

Blood samples and ascetic fluid collection

Animals were monitored regularly for alterations in body weight, for the development of any signs of toxicity and mortality. Body weight was registered at day 0, 3, 6, 9 and day15 (day of animal sacrifice). 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 for determination of hematological parameters. Organs included liver, spleen, kidney, heart and lung were collected, rinsed, with saline, dried and weighted then fixed in 10% formalin for histopathological examination. Ascetic fluid (if present) volume was measured, and tumor cells were enumerated.

Methods

Change in Body Weight

Body weight was registered for each mouse at beginning of the experiment and every 3 days till day 15 (day of animal sacrifice). The percent change in body weight was calculated using the formula described by Kuttan et al. [15]:

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

Where W_1 is the body weight of each mouse at the start of the experiment and W_2 is the body weight of animal at the end of observation.

Mean survival time and percentage increase in life span

Animal survival time was recorded and expressed as mean survival time (MST) in days and the percentage increase in lifespan (% ILS) was calculated using the equations described by Thalla et al. [16]:

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

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

Measurement of ascetic fluid volume and EAC cells viability

The developed ascetic fluid was obtained from each mouse in EAC-bearing control animals and PACBpy-Pt treated group under aseptic conditions and was measured. The fluid was centrifuged to collect EAC cells. The cells were tested for viability by trypan blue using hemocytometer.

Cell count = (No. of cells x Dilution) / Area x Thickness of liquid film

Measurement of relative organs weight

On the 14th day, animals were overnight fasted and were sacrificed. Vital organs including liver, spleen, kidneys, heart and lung were autopsied and examined macroscopically for any lesions or abnormalities. Organs were weighted, after washing with saline and drying. The relative organs weight of each animal was then calculated [17] as follows:

Relative organ weight = (Absolute organ weight / body weight of mice on the day of sacrifice) ×100

Hematological parameters

Blood was obtained from tail vein of the mice under light ether anesthesia using heparinized micro-capillaries. Red blood cells (RBC_S), white blood cells (WBC_S) 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 of alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP); in addition to levels of: total proteins, albumin, globulin, urea and creatinine. Determination of serum glucose, lipid profile (triglycerides, cholesterol and very low density lipoproteins) and cardiac profile (lactate dehydrogenase, creatine kinase and isoenzymes of creatine kinase) were also carried out. All the biochemical parameters were determined using specific kit for each parameter.

Antioxidants activities

Determination of total antioxidant capacity (TAC), lipid peroxides (as MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione-S transferase were carried out using specific kit for each enzyme.

Histopathological examinations

Theheart, liver, kidney, lung and spleen were removed; slides prepared, staining was done and viewed under light microscope of 40x magnification.

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 (η2) and Cohen's d tests were used to estimate the effect of size that describes the proportion of total variability attributable to a variable. Independent-Samples t Test (t) was performed for comparing means for EAC and 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.

RESULT

Cytotoxicity study

Changes of body weight

At the beginning of the experiment (zero days), the mean values of body weight for both EAC bearing mice and treated groups were matched with normal group mean value. Significant increase in body weight was noted in EAC group after 9 and 15 days compared to normal group. PCABPY-Pt treated mice bearing tumor showed significantly decreased body weight compared to normal mice group after 3, 6, 9 and 15 days of treatment, although the body weight was matched with those for normal mice group at the beginning of the experiment. The changes in body weight of the studied groups during 15 days of experiment are shown in Table 1. The percent change in body weight between day zero and day 15 is shown in Fig. 1. Mice inoculated with EAC tumor cells showed a maximum gain in body weight that amount to 23%. Treatment by PCABPY-Pt complex after tumor inoculation showed 15% gain in body weight at the end of experimental period compared to zero day.

Mean survival time (MST) and life span (%ILS)

MST and %ILS were calculated for both PCABPY-Pt and treated groups using normal mice group as a control for PCABPY-Pt, while EAC group was a control for treated group. Treatment with 80 mg PCABPY-Pt / kg after EAC inoculation improved the mean survival time of mice which in turn resulted in prolonged life span (58 %). Normal mice injected with a dose equivalent to 80 mg/kg body weight of PCABPY-Pt showed reduction in their MST resulting in decreased life span by 40% (Table 2).

Table 1: Body weight (B.Wt) during experimental period in all studied groups

Groups / Parameters	Normal	EAC	Vehicle	PCABPY-Pt	Treated
B.Wt at 0 day *p **p	30.3±0.32 - -	31.3±0.42 NS -	32.5±0.45 0.001 NS	32.4±0.33 0.001 NS	31.2±0.40 NS NS
B.Wt after 3days *p **p	31.8±0.35 - -	35.4±0.50 0.001 -	34.9±0.60 0.001 NS	36.05±0.43 0.001 NS	34.7±0.36 0.001 NS
B.Wt after 6days *p **p	32.4±0.30 - -	36.3±0.41 0.001 -	36.7±0.55 0.001 NS	35.3±0.39 0.001 NS	34.0±0.31 NS 0.01

B.Wt after 9days *p **p	32.8±0.33 - -	37.4±0.54 0.001 -	36.5±0.54 0.001 NS	34.5±0.38 0.04 0.001	34.9±0.30 0.01 0.01
B.Wt after 12days *p **p	33.1±0.28 - -	38.5±0.43 0.001 -	35.9±0.47 0.001 0.001	34.5±0.36 NS 0.001	35.8±0.37 0.001 0.001

*p: values vs. normal group mean value; **p: values vs. EAC group mean value; NS: non-significant; p value > 0.05 is significant.

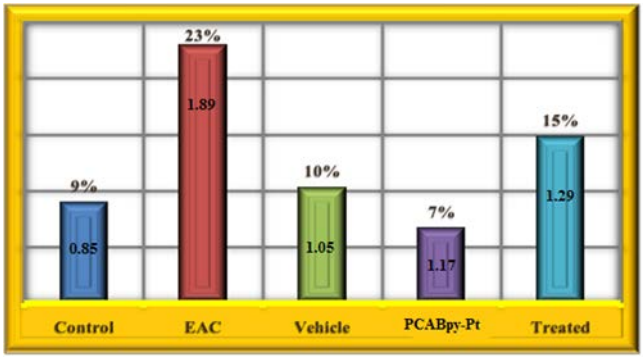

Fig. 1.The means and standard errors of percent changes in body weight.p value vs. control group: EAC (0.001); vehicle (NS); PCABPY-Pt (NS) and treated (0.001).

Ascetic fluid, viable and non-viable cell count

Ascetic fluid, viable cell count and percentage viable cell were significantly increased in tumor bearing mice group. Injection of 80 mg/kg body weight of PCABPY-Pt complex to EAC cells inoculated mice had significantly increased non-viable cell count due to inhibition of ascetic fluid accumulation (Table 3).

Hematological profile

Hematological parameters of untreated tumor bearing animals were significantly altered compared to healthy normal animals (RBCs and Hb contents were decreased while WBCs were increased, $p < 0.001$). Intraperitoneal injection of Pt-complex to EAC inoculated mice had significantly improved hematological parameters, compared to non-treated EAC group reaching values comparable to healthy control mice in case of Hb (Table 4).

Biochemical studies

liver and kidney Functions:

Results revealed significant elevation in the activities of ALT, AST, ALP and globulin mean values with reduction in the mean values of total proteins and albumin in EAC bearing mice compared to normal mice group. On the other hand, results revealed significant elevation in serum urea in EAC group compared to normal healthy control, treatment with Pt-complex had almost ameliorate the changes induced by EAC inoculation (Table 5).

Table 2: Mean survival time (MST) and life span (%ILF) in all groups.

Groups / Parameters	Normal	EAC	Vehicle	PCABPY-Pt	Treated
MST(Days)	120	26.5	120	71	42
ILS (%)	-	-	-	- 40	58

Table 3: Ascetic fluid volume and EAC cell viability in tumor bearing and treated groups.

Groups / Parameters		EAC	Treated
Ascetic fluid (ml)		2.50±0.10	0.00*
Cell count	Viable ($x10^8$cells/mouse)	4.13±0.52	0.23±0.03*
	Non-viable ($x10^8$cells/mouse)	0.43±0.05	4.31±0.26*
Percentage of viable cells (%)		89.01±1.04	5.00±0.66*
Percentage of non-viable cells (%)		10.9±1.04	95.0±0.66*

*p value ≤ 0.001.

Table 4: Hematological parameters in all studied groups (X ± SE).

Groups / parameters	Normal	EAC	Vehicle	PCABPY-Pt	Treated
Hb(g/dl) *p **p	13.5±0.19 - -	10.7±0.08 0.001 -	13.7±0.08 NS 0.001	14.4±0.13 NS 0.001	13.5±0.52 NS 0.001

RBCs(x10^{6}cell/uL) *p **p	8.6±0.04 - -	6.5±0.05 0.001 -	8.0±0.04 0.01 0.001	8.5±0.08 NS 0.001	7.7±0.21 0.001 0.001
WBCs(x10^3/uL) *p **p	6.8±0.17 - -	12.4±0.14 0.001 -	5.1±0.22 0.001 0.001	7.5±0.28 NS 0.001	4.4±0.20 0.001 0.001

Hb: hemoglobin; RBCs: red blood cells; WBCs: white blood cells. *p value vs. control group; **P value vs. EAC group. NS: non-significant; P value < 0.05 is significant

Table 5: Liver and kidney functions for the studied groups

Parameters \ Groups	Normal	EAC	Vehicle	PCABPY-Pt	Treated
ALT (U/L) ***P** ****P**	71.1±5.23 - -	252±32.01 0.001 -	71.9±2.48 NS 0.001	78.2±5.32 NS 0.001	57.0±0.86 NS 0.001
AST (U/L) ***P** ****P**	260±0.69 - -	346±35.06 0.001 -	259±4.79 NS 0.001	266±4.38 NS 0.001	249±5.02 NS 0.001
ALP (U/L) ***P** ****P**	45.6±0.63 - -	82.2±2.23 0.001 -	57.2±1.94 NS 0.001	40.5±1.37 NS 0.001	31.3±0.99 0.001 0.001
GGT (U/L) *P **P	5.00±0.00 - -	5.00±0.00 0.001 -	4.9±0.05 NS 0.001	4.6±0.11 NS 0.001	5.00±0.00 0.04 0.001
Total Proteins (g/dl) ***P** ****P**	5.1±0.09 - -	4.3±0.04 0.001 -	4.4±0.08 NS 0.001	5.3±0.02 NS 0.001	4.9±0.04 0.001 0.001
Albumin (g/dl) ***P** ****P**	2.5±0.05 - -	2.0±0.03 0.001 -	2.2±0.01 NS 0.001	2.5±0.01 NS 0.001	2.2±0.02 0.001 0.001
Globulin (g/dl) ***P** ****P**	2.7±0.05 - -	2.3±0.02 0.001 -	2.2±0.07 NS 0.001	2.8±0.02 NS 0.001	2.6±0.02 NS 0.001
Urea (mg/dl) ***P** ****P**	60.8±1.38 - -	82.6±2.27 0.001 -	64.6±2.77 NS 0.001	64.8±1.07 NS 0.001	67.4±2.31 NS 0.001
Creatinine(mg/dl) ***P** ****P**	0.28±0.01 - -	0.32±0.02 NS -	0.24±0.01 NS 0.001	0.20±0.01 0.001 0.001	0.24±0.01 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.

Serum glucose and lipid profile

EAC group had significantly elevated lipid parameters with the exception of LDL and HDL mean values compared to control group. Treated mice group had significantly improved lipid Profile than EAC group. Mean values of serum glucose and lipid profile parameters were comparable to then matched values in control animals group. Administration Pt-complex to healthy animals did not affect lipid parameters, however, elevated blood glucose was observed (Table 6).

Cardiac Profile

Significant elevation in LDH, CK (total) and CK-MB markers induced by inoculation with EAC cells was noted. No significant improvement in LDH by treatment was indicated. Meanwhile, CK and CK-MB mean values showed significant reduction in treated animals (Table 7).

Table 6: Serum glucose and lipid profile for the studied groups

Parameters \ Groups	Control	EAC	Vehicle	PCABPY-Pt	Treated
Glucose mg/dl *P **P	130±4.90 - -	123±1.29 NS -	125±6.49 NS NS	148±2.46 0.001 0.001	126±1.21 NS NS
Triglycerides mg/dl *P **P	102±5.90 - -	157±3.83 0.001 -	101±8.17 NS 0.001	104±5.62 NS 0.001	104±3.35 NS 0.001
CHOL mg/dl *P **P	99.6±4.14 - -	113±3.92 0.03 -	98.4±1.68 NS 0.02	98.6±1.39 NS 0.01	107±1.38 NS NS

VLDL mg/dl *P **P	20.4±1.16 - -	31.6±0.78 0.001 -	20.4±1.65 NS 0.001	21.0±1.13 NS 0.001	21.2±0.65 NS 0.001
LDL mg/dl *P **P	14.8±0.55 - -	17.4±1.04 NS -	15.3±0.50 NS NS	15.3±0.73 NS NS	7.2±0.28 0.001 0.001
HDL mg/dl *P **P	76.2±3.97 - -	68.6±1.91 NS -	62.6±2.99 0.01 NS	70.8±1.07 NS NS	79.0±1.67 NS NS

*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.

Table 7: Serum Cardiac Profile for the studied groups

Groups / parameters	Normal	EAC	Vehicle	PCABPY-Pt	Treated
LDH(U/L) *p **p	478±17.0 - -	1277±75.6 0.001 -	500±21.4 NS 0.001	511±80.3 NS 0.03	892±7.2 0.001 0.01
CK –total (U/L) *p **p	76.5±1.74 - -	282±21.6 0.001 -	141±3.05 0.001 0.001	96.0±4.99 NS 0.001	114±9.44 0.001 0.001
CK-MB (U/L) *p **p	107±2.24 - -	154±4.39 0.001 -	179±9.80 NS 0.001	132±3.33 NS 0.001	98.6±4.88 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.

Antioxidant Activities

Results demonstrated significant reduction in total antioxidant capacity, SOD and CAT of animals inoculated with EAC, in addition to elevated MAD in same animal group. In relation to control mean values, normal mice injected with the Pt-complex had significantly higher mean for total antioxidant capacity and GST, with decreased MDA and non-significant variation in SOD and CAT activities mean values (Table 8).

Table 8: Antioxidant Activities in all studied groups

Groups / parameters	Normal	EAC	Vehicle	PCABPY-Pt	Treated
TAC (mmol/L) *p **p	0.36±0.00 - -	0.11±0.01 0.001 -	0.38±0.01 NS 0.001	0.60±0.05 0.001 0.001	0.42±0.01 NS 0.001
MAD (mmol/ml) *p **p	19.3±0.14 - -	106±1.46 0.001 -	19.5±0.13 NS 0.001	14.2±0.41 0.001 0.001	11.6±0.23 0.001 0.001
SOD (U/ml) *p **p	169±2.14 - -	116±7.22 0.001 -	160±2.69 NS 0.001	164±6.44 NS 0.001	145±2.73 0.001 0.001
CAT (U/ml) *p **p	943±2.80 - -	735±12.27 0.001 -	936±3.94 NS 0.001	948±3.28 NS 0.001	935±5.42 NS 0.001
GST(U/L) *p **p	1123±8.04 - -	878±21.9 NS -	1194±26.4 NS NS	2770±137 0.001 0.001	954±5.06 NS NS

*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.

Histopathological Examinations

The histopathology studies also revealed the relatively less toxic of (80 mg/kg) PCABPY-Pt complex as compared to control and EAC group when viewed under light microscope of magnification 40x. The histopathology of the heart, liver, kidney, lung and spleen tissues of normal, EAC, vehicle, PCABPY-Pt complex andtreated groups are shown in **Fig.2.Histopathology of heart:** the cardiac histopathology of EAC bearing mice indicates dilated spaces between myocytes cells, nuclear myocardial pyknosis, karyolysis and wide dilated blood vessels, few inflammatory cells are also seen. While, the cardiac tissue of treated group has less dilated space between myocytes cells, less nuclear myocardial pyknosis, less karyolysis and very few dilated blood vessels, no inflammatory cells. **Histopathology of liver:** liver tissue of EAC group showed increased number of Kupffer cells, congested central vein with hemorrhage and dilated congested blood sinusoids. Degenerated necrotic hepatocytes and mildly atypical hepatocytes were noticed; many inflammatory cells are also seen.On the other hand, liver tissue from the EAC bearing mouse treated with Pt-complex showed few

number of Kupffercells, less congested central vein with hemorrhage and less congested blood sinusoids. Few degenerated necrotic hepatocytes with no atypical hepatocytes and few inflammatory cells. **Histopathology of kidney:** renal tissue of EAC group showed pathological changes such as the necrotic degenerated renal glomeruli with interstitial hemorrhage, focal necrotic degenerated tubular cells and inflammatory cells. **Histopathology of lung:** lung tissue from the EAC bearing mouse showed markedly congested interstitial vessels with hemorrhage and mixed inflammatory cells including macrophages. Examination of lung tissue from the EAC bearing mouse treated with Pt-complexshowed less congested interstitial vessels with little hemorrhage and few inflammatory cells. **Histopathology of spleen:** spleen tissue of EAC group that have nearly disturbed splenic architecture showing markedly congested splenic sinusoids with increased numbers of littoral cells. Many multinucleated giant cells and many inflammatory cells were detected. Also, the spleen tissue of treated group showed congested splenic sinusoids with few numbers of littoral cells and inflammatory cells. No multinucleated giant cells wereidentified.

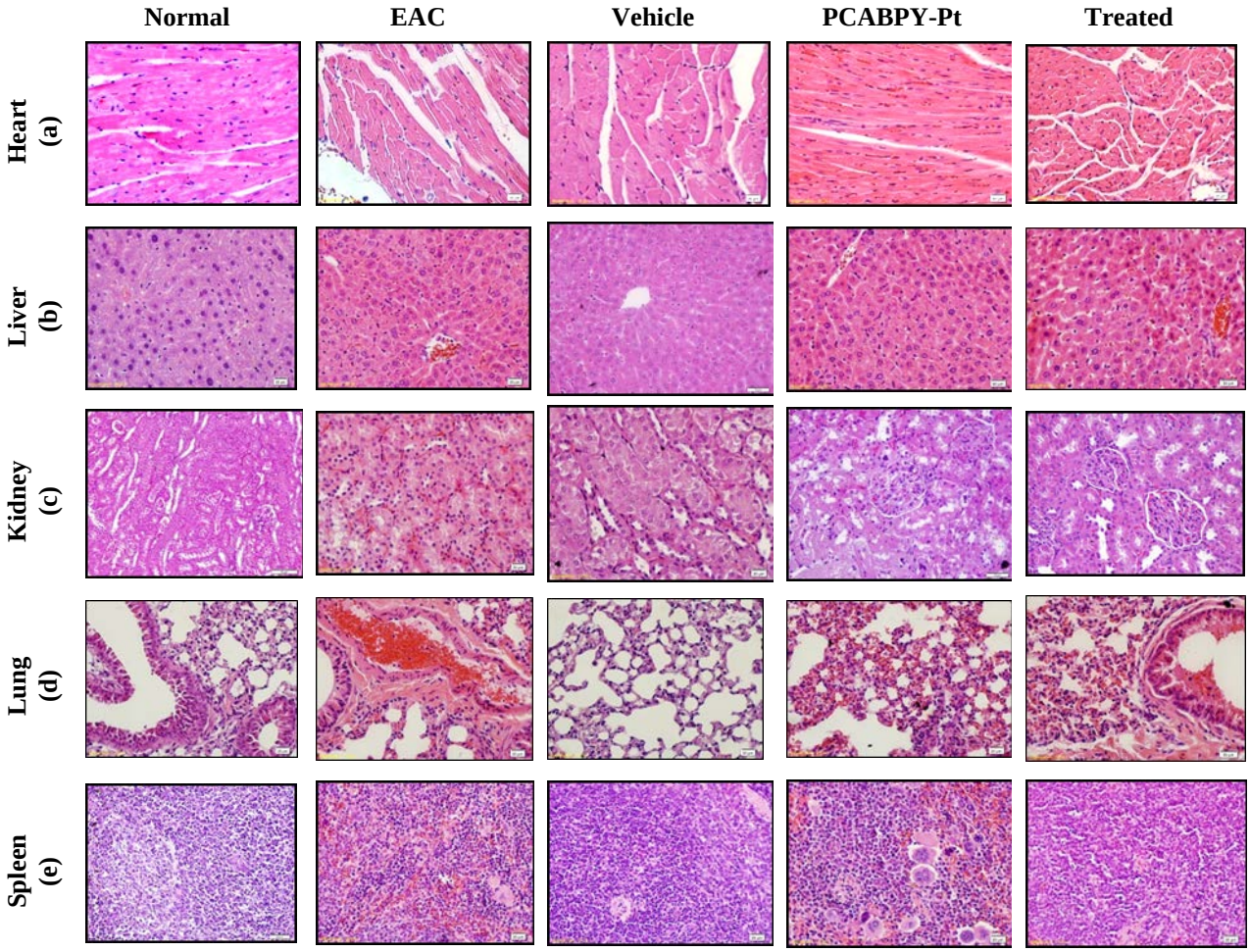

Fig. 2: ***Histopathological Examinations (40x).***

DISCUSSION

The present study aimed to evaluate the antitumor activity beside anti-oxidative stress of PCABpy-Pt complex against EAC cells induced in male albino mice. Mice inoculated with EAC cells had significantly increased body weight after 15 days of tumor cells inoculation (~ 23 %) compared to normal mice (~ 9 %), $p \leq 0.001$, which could be attributed to tumor burden. The Ehrlich tumor cells are aggressive rapidly growing cells [18] and are able to grow in different mice strains [19]. The implantation of EAC cells induces local inflammatory reaction with increasing vascular permeability resulting in edema, cellular migration and a progressive ascetic fluid formation [20]. Treating EAC inoculated mice with PCABpy-Pt complex had prevented the accumulation of ascetic fluid resulting in 15% weight gain at the end of experiment. Treatment also decreased the number of viable EAC cells and increased the numbers of non-viable cells, indicating cytotoxic effect of the drug.

Treating EAC inoculated mice with PCABpy-Pt resulted in increasing the mean survival time (42 days) compared to non-treated EAC control group (26.5 days), which in turn resulted in prolonged life span by 58 %. One of the reliable criterion for judging the value of anticancer drug is the prolongation of animal life span [21], where an enhancement of life span up to 25 % or more was considered as an effective anticancer [22].

The tumor induction had significantly decreased RBCs and hemoglobin contents and increased WBCs count by nearly 24 %, 21% and 82 % respectively. The anemia encountered in tumor bearing mice is mainly due to a reduction in RBCs or hemoglobin percentage which may be due to iron deficiency or due to hemolytic ormyelopathic conditions [23, 24]. Recovery of the hemoglobin content, RBCs and WBCs count observed by treatment indicated the protective action of PCABpy-Pt complex on the heamopoietic process. The present study indicated that EAC cells inoculation had significantly induced hepatotoxicity in mice as manifested by elevated AST, ALT and ALP activities and reduced total proteins, mainly albumin, indicating hepatocellular damage by EAC cells. It is well known that increased activities of liver transaminases are characteristics of liver diseases, and very high levels are observed in toxic hepatitis. Chronic liver diseases are also associated with elevated ALP [25]. Moreover, plasma proteins gives useful information in chronic liver diseases especially advanced liver diseases. Damaged hepatoocytes of EAC bearing mice might be due to cytotoxic agent In the current work, the damaging effect observed by EAC inoculation was improved by treatment with antitumor PCABpy-Pt complexitself or due to its toxic metabolites [26, 27].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 [28, 29].

Lipids were deranged in EAC bearing mice, where significantly elevated TAG, cholesterol and VLDL levels were noted. Elevated serum cholesterol and TAG in EAC bearing mice was reported by many studies [30-34]. Disturbances in hepatocellular function might results in defect in the removal of cholesterol from the circulation. On the other hand, hypertriglyceridemia might be due to biochemical changes in diseased liver affecting transport of TAG out of the liver [35]. Increased mobilization of fats from the adipose tissue to the blood stream might be also involved in elevated TAG and consequently synthesis of VLDL by liver [36]. Treatment of EAC bearing mice with PCABpy-Pt complex reduced, to a great extent, the deleterious effect of tumor on lipid components.

Free radicals, most importantly reactive oxygen species (ROS), are known to stimulate the development of cancer, and high levels of H_2O_2 are extensively produced by a variety of human tumors [37]. Both SOD and CAT are known to be involved in the clearance of superoxide ions and H_2O_2 [38, 39]. Increased production of ROS enhances oxidative stress which leads to macromolecules damage; such as lipid peroxidation [40].The lipid peroxide formed in the primary site would be transferred through circulation and provoke damage by propagating the process of lipid peroxidations [40]. MDA was known to be elevated in cancer tissues [41] and its elevated levels is related to tumor progression [42]. Moreover, A has high cytotoxicity and inhibitory action on protective enzymes that makes tumor promoter and co-carcinogenic [43]. In the present investigation, inoculation of EAC tumor cells to healthy mice had reduced the antioxidant status of the animals. Total antioxidant capacity, SOD and CAT activitieswere 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, PCABpy-Pt complex can restore the antioxidants status of the treated mice. Elevated glutathione-S-transferase (GST) was clear by i.p. injection of the complex to normal mice. GST is a detoxification enzyme that is involved in direct detoxification as well as acting as an inhibitor of the MAP kinase pathway, therefore protects cellular macromolecules from attack by electrophiles. GST catalyze the conjugation of glutathione (GSH) to different endogenous and exogenous electrophilic compounds, which is the first step in the mercapturic acid pathway that leads to elimination of toxic compounds [44].Therefore, elevated GST might be involved in eliminating the side effects, due to drug injection to healthy animals.

CONCLUSION

The overall finding of this study confirms the therapeutic effect of the PCABpy-Pt complex against EAC cells subcutaneously inoculated to mice. The complex had successfully inhibited ascetic fluid formation, decreased tumor cell viability, and therefore reduced the tumor burden. The complex had significantly protected the vital organs against damage induced by EAC cells inoculation. Improvement of the antioxidant status might be responsible for the potent antitumor activity of the drug.

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