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Optimizing the antitumor efficacy of cisplatin low doses by the co-treatment with ethylenediamine tetra acetic acid in EAC-bearing mice

Yosery E. El-Bolkiny[1, *], Fatehyia Makboul[2], Mona M. Elwan[1, *]

[*,1]Zoology Department, Faculty of Science, Tanta University, Egypt, [2] Laboratory Department, Swiss Governorate, Ministry of Health, Cairo, Egypt

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Corresponding authors:

1. Yosery E. El-Bolkiny, Ph.D.
Zoology Department, Faculty of Science, Tanta University, Egypt
E-mail: bolkiny61@yahoo.com
Mobile: (+2) 01021850601

2. Mona M. Elwan,Ph.D.
Zoology Department, Faculty of Science, Tanta University, Egypt
E-mail: monaelwan287@yahoo.com
Mobile: 01091818724

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ABSTRACT

Cancer disease remains one of the most cause of death worldwide. The drawbacks that were associated to the chemotherapy treatments are impetus for finding more efficacious, and better tolerated new drugs. This study was conducted to optimize the low doses of cisplatin (Cis) antitumor effect by the co-treatment of ethylenediamine tetra acetic acid (EDTA) in EAC-bearing mice. Sixty-four female CD-1 mice were divided into eight groups (n = 8) as follows: Gp1 was a negative control. Gp2 had inoculated with 1×106 EAC-cells/mouse interperitoneally (i.p.). Gp3, Gp4 and Gp5 had inoculated with the same number of EAC-cells as in Gp2, then injected with 0.125, 0.250 or 0.5 mg/Kg of Cis i.p, respectively. Gp6, Gp7 and Gp8 had inoculated with EAC-cells as in Gp2, then injected with the low doses of Cis as in Gp3- G5 in combination with EDTA (50 mg/Kg), respectively. At day 14, all groups were bled to collect sera for biochemical assessments. The tumor ascites was collected for determining the total tumor volume and total tumor counts. Liver tissues were harvested for histopathological investigations. The results showed that co-treatment of EDTA (50 mg/Kg) along with the low doses of Cis (0.125, 0.25 or 50 mg/Kg) led to significant reduction in tumor volume, tumor cells count, improved the liver, kidney functions, and antioxidant status. Among all the treated EAC-bearing mice groups, mice that had a combined treatment with Cis (0.5 mg/Kg) and EDTA (50 mg/Kg) showed the highest antitumor effect with highly improvements in the liver functions and architectures.

Keywords:
Antitumor, Cisplatin, Ethylenediamine tetra acetic acid, EAC-bearing mice.

1. Introduction

Cancer is considered the 2nd most lethal disease after cardiovascular disease in humans worldwide (Bray et al., 2018). Classical chemotherapeutic and radio-therapeutic agents are cytotoxic with deleterious effect on normal tissues (Olga et al., 2021). Cisplatin (Cis) is a chemotherapeutic drug used for the treatment of different types of cancer. Cis exhibited its antitumor effect *via* generating DNA adducts, which in turn leads to cell cycle arrest and initiating apoptotic signaling pathway (Dasari and Tchounwou, 2014). Hepatotoxicity, nephrotoxicity, and cardiotoxicity are associated with Cis-treatment (El-Sawalhi and Ahmed, 2014). It was reported that Cis-induced multi-organs toxicity such as ototoxicity, myelosuppression, and reproductive toxic effects (Dasari and Tchounwou, 2014). In addition, Cis treatment led to a decrease in antioxidant defense system through reactive oxygen species (ROS) generation (Kart et al., 2010).

Combination chemotherapy with Cis is the basis of treatment of several cancer types to overcome the bad mechanisms of Cis resistance and its adverse toxicity (Gottesman et al., 2002). For

instance, the presence of quercetin or ibuprofen with Cis ameliorates the nephrotoxicity, enhanced antitumor activity, and accelerates the apoptosis of cancer cells (Li et al., 2016). The combination of Cis, paclitaxel, and 5-fluro-uracil is an active and tolerable therapy in Chinese patients with an advanced gastric and esophagogastric junction adenocarcinoma (Kim et al., 1999). Combination of uracil plus futrafur (UFT), and Cis is shown to be an effective therapy for the treatment of non-small cell lung carcinoma (Ichinose et al., 2000). Cis plus gemcitabine is an appropriate option for advanced biliary cancer treatment (Valle et al., 2010).

Iron plays a significant role in the metabolism of cancer cells. The neoplastic cells exhibit enhanced vulnerability to iron by substantially increasing the demand for the microelement in comparison with healthy cells (Szlasa et al., 2021). Due to the high concentration of iron (II) ions in the cytoplasm of the cancer cells enhances the ferroptosis by increasing the lipid peroxidation process (Xie et al., 2016). As iron chelators such as EDTA were developed to treat iron overload, these agents are being repurposed to treat cancers (Lane et al., 2014). In the recent decade, the role of iron chelates in targeting the growth of the tumor was extensively examined (Szlasa et al., 2021). Two main strategies of iron chelating action for cancer treatments are considered. The 1st includes depletion of iron from cancer cells by the inhibition of cellular iron uptake. The 2nd focuses on the application of iron chelates to facilitate the redox cycling of iron, to generate cytotoxic ROS within tumor (Torti and Torti, 2013). Iron chelators could inhibit the ribonucleotide reductase, which limits the iron-dependent enzymes for DNA synthesis or on excitation of cell cycle arrest (Lane et al., 2014).

Ethylenediamine tetra-acetic acid (EDTA) used to cure myocardial, atherosclerosis, and occlusive arterial disease (Sulit et al., 2020). EDTA reduces the release of free radicals from oxidation/reduction reactions (Vidyalaya et al., 2016). EDTA has been tested *in vitro* as an anticancer agent (Feril et al., 2017; El-Naggar et al., 2021). Previous study reported that EDTA did not show any antitumor activities and did not alter Cis-antitumor effect in Ehrlich ascetic carcinoma (EAC) bearing mice (El-Naggar and El-Said, 2020). Similar study showed that the treatment with liposomes loaded with EDTA alone did not show antitumor effects, however, liposomes loaded with EDTA and doxorubicin could significantly reduce drug toxicity without altering the antitumor activity (Song et al., 2014). Furthermore, intra-tumoral EDTA injection enhanced the antitumor efficacy of Cis on colon cancer in rats (Duvillard et al., 2004). Therefore, this study was conducted to determine the optimal combination of the low doses of Cis with EDTA to maximize its antitumor efficacy in EAC-bearing mice.

2. Materials and Methods

Chemicals

Cisplatin (Cis) and ethylenediamine tetra-acetic acid (EDTA) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Alanine amino transferase (ALT), aspartate amino transferase (AST), creatinine, urea, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) kits were purchased from Bio-diagnostic Company, Egypt.

Ehrlich ascites carcinoma (EAC) cells

EAC cells were collected from the tumor bearing mice, which purchased from the National Cancer Institute (NCI, Cairo, Egypt). The viable and dead cells were counted using routine trypan blue method. The total viable EAC-cells were calculated as follows: Mean number of unstained cells × dilution $\times 10^4$. The number of EAC-cells was adjusted at 1×10^6 cells/mouse for i.p inoculation.

Mice and EAC-cells inoculation

Female CD-1 mice (20 ± 3 g) were obtained from National Research Center (NRC, Cairo, Egypt). Mice were kept for a week in plastic cages for adaptation. The temperature and relative humidity were about 22 ± 1ºC and 55 ± 5%, respectively. Mice were subjected to normal light-dark (day/night) cycle and then handled according to the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University (Protocol No.: IACUC-SCI- TU 0186). Mice were given drinking tap water and normal experimental pelleted animal food *ad libitum*. Tumor cells (EAC) were i.p inoculated with 1×10^6 cells/mouse. At the end of the experiment, the

volume of ascetic fluid was determined by needle (18–22 gauge) aspiration. Withdrawal of ascetic fluid was performed under aseptic conditions.

Experimental design

Sixty-four female CD-1 mice were divided into eight groups (n = 8) as follows: the first group (Gp1), mice were i.p injected with 200 µl saline. Mice in Gp2 were i.p inoculated with 1×10^6 EAC-cells/mouse. Gp3, Gp4 and Gp5 were inoculated with the same number of EAC-cells as in Gp2, then injected with 0.125, 0.250 or 0.5 mg/Kg of Cis (i.p), respectively. Gp6, Gp7 and Gp8 were inoculated with EAC-cells as in Gp2, then injected with the low doses of Cis (i.p) as in Gp3, Gp4 and G5 in combination with EDTA (50 mg/Kg), respectively. The treatment protocol of Cis low doses and EDTA were daily injected for consecutive 6 days (D1 - D6). On day 14 and under appropriate anesthesia (Anahal, Isoflurane 100%), all mice were bled through the orbital plexus to collect blood samples for biochemical assessments and then were dissected to collect the tumor ascites, then liver tissues were harvested, and small pieces were fixed in buffered formalin for histopathological investigations.

Determination of the total body weight changes (% b.wt change)

Mice were weighed at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of body weight changes **(**% b.wt) was calculated as follows:

The % b.wt change = [(final b.wt – initial b.wt) / initial b.wt] × 100.

Measurements of tumor parameters:

EAC-bearing mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The tumor parameters were measured in EAC bearing mice in respect to; total tumor volume (T.T.V), total tumor cell counts (T.T.C), total live and dead EAC-cells (T.L.C, T.D.C), respectively. The total volume of the fluid was measured in a graduated centrifuge tube. To count the total EAC-cells, the ascitic fluid was diluted and then the live and dead cells were counted using trypan blue stain exclusion technique (Kundusen et al., 2011).

Serum and tissue samples preparation:

At the end of the treatment protocol, mice in each group used for blood sampling from retro-orbital venous plexus under light anesthesia using heparinized microhematocrit tubes. Blood samples were allowed to clot for 2 hours at room temperature or overnight at 2-8°C, and then centrifuged at approximately 1000 × g (or 3000 rpm) for 15 minutes. Sera were immediately separated, and aliquot then stored at -20°C for biochemical analysis.

Liver histological examinations:

During dissection, two small pieces of each mouse liver were taken out; one piece to prepare tissue homogenate and the other for histological investigation. that immediately fixed in 10% neutral buffered formalin. After washing to remove the excess of fixative, the tissue samples were dehydrated in ascending grades of ethyl alcohol, cleared by xylene, and embedded in paraffin wax. Sections of 5 µm thickness were mounted and stained with routine hematoxylin and eosin stains for histological examination (Bancroft and Gamble, 2008). For preparing tissue homogenate, liver pieces were quickly removed, and all surrounding connective tissues were removed carefully. Then after, 10% (W/V) homogenate was prepared by splendid 0.5 g of liver tissue in 5 ml in buffer saline to prepare 10% tissue homogenate. Supernatants were carefully separated for oxidative stress biomarkers estimation.

Determination of biochemical parameters:

Alanine transaminase (ALT) and aspartate transaminase (AST) (Reitman and Frankel 1957) (Cat. No. AT103445), creatinine (Schirmeister et al., 1964) (Cat. No. CR1250) and urea (Cat. No. UR2110) (Friedman and Young 1997) were determined by colorimetric methods according to the manufacturer's protocol. Activities of SOD (Cat. No. SD2521), CAT (Cat. No. CA2517), GSH (Cat. No. GR 25 11), and MDA (Cat. No. MD2529) levels were assessed in the liver tissue using Bio-diagnostic Company kits according to the manufacturer's protocol**.**

Statistical analysis:

The data were expressed as mean ± standard deviation ($X \pm SD$). Comparison between groups was carried out using one-way ANOVA. If there is a significant difference between means, Tukey's post-hoc comparisons among different

groups were performed. *P* values < 0.05 were statistically significant. Data and statistical analysis were performed using Excel 2013 (Microsoft Corporation, USA) and Minitab version 18.

3. Results

Co-treatment with EDTA reduced the change % in the body weight of Cis-treated EAC-bearing mice:

After two weeks of the beginning of the experiment, the change % of b.wt in normal control group was 18.78%, while, it was 32.5% in EAC-bearing mice. In EAC-bearing mice that treated with the low doses of Cis (0.125 or 0.250, 0.05 mg/Kg), the change % in the b.wt were 28.12%, 17.77%, and 14.81%, respectively. In EAC-bearing mice that treated with the low doses of Cis (0.125, or 0.250, 0.05 mg/Kg)/EDTA (50 mg/Kg), these percentages were reduced to 8.59%, 2.93%, and 0.74%, respectively (Figure 1), i.e. EDTA reduced the body weight in EAC-mice.

EDTA increased the antitumor efficacy of Cis low doses in EAC- bearing mice:

Treatment of EAC-bearing mice with the low dose of Cis (0.125 mg/Kg) led to a significant reduction in the total tumor volume, total tumor cells count, and live tumor cells count ($p \leq 0.05$). In EAC-bearing mice that treated with the low dose of Cis (0.250 mg/Kg), the total tumor volume, total tumor cells count, and live cells tumor count were reduced more than their corresponding values in the group of EAC-bearing mice that treated with Cis (0.125 mg/Kg) alone (Table 1). EAC-bearing mice that treated with the low dose of Cis (0.5 mg/Kg) showed significant reduction in the total tumor volume, total tumor cells count, viable EAC cells and increased the number of dead EAC-cells when compared to their corresponding values in EAC-bearing mice ($p < 0.001$). EDTA co-treatment (50 mg/Kg) either with the low doses of Cis (0.125, 0.25 or 50 mg/Kg) increased the reduction in the total tumor volume, total tumor cells count, and viable tumor count in EAC-bearing mice significantly when compared to EAC-bearing mice that had treated with the low doses of Cis-alone (Table 1).

EDTA ameliorated the hepato-renal toxicity of Cis low doses in EAC-bearing mice:

The results in Table 3 showed that the liver transaminases (ALT and AST) activities in EAC-bearing mice were increased significantly when compared to their values in the naïve mice ($p \leq 0.05$). ALT and AST activities in EAC-bearing mice that treated with the low doses of Cis (0.125, 0.250 or 50 mg/Kg) were decreased when compared to their values in EAC-bearing mice alone. Co-treatment of EDTA (50 mg/Kg) with low doses of Cis (0.125, 0.250 or 50 mg/Kg) led to improvement in the liver ALT and AST activities. The levels of creatinine and urea in EAC-bearing mice were significantly increased when compared to their levels in the control group ($p \leq 0.05$). Treatment of EAC-bearing mice with the low doses of Cis (0.125, 0.250 or 50 mg/Kg), however, led to significant decrease in their levels compared to EAC-bearing mice alone ($p \leq 0.05$). EAC-bearing mice that treated with combinations of the low doses of Cis (0.125, 0.250 or 50 mg/Kg) and EDTA (50 mg/Kg) showed significant improvements in the kidney function as evidenced by decreasing the levels of creatinine and urea levels comparing with EAC-bearing mice that only treated with the low doses of Cis

EDTA augmented the antioxidants/oxidant's status in low doses Cis-treated EAC-bearing mice:

Compared to naïve mice, the activities of SOD, CAT, and GSH level were decreased while the level of MDA was increased significantly in EAC-bearing mice ($p \leq 0.05$). on the other hand, treatment of EAC-bearing mice with the low doses of Cis (0.125, 0.250 or 50 mg/Kg) led to increase the activities of SOD, CAT, and GSH level while decrease the MDA level significantly when compared to EAC-bearing mice ($p \leq 0.05$). Co-treatment of the low doses of Cis (0.125, 0.250, 50 mg/Kg) with EDTA (50 mg/Kg) led to further increase in SOD, CAT activities, and GSH level, concomitant with a further decrease in the MDA level when compared to EAC-bearing mice that treated with the low doses of Cis alone (Table 4).

Cis/EDTA ameliorated the histopathological changes in the liver tissues of EAC-bearing mice;

As shown in figure (2A), light microscopic examination of the liver sections of mice in the control group showed normal hepatic

architecture, normal central vein and radiating polygonal hepatocytes. The liver strands were alternating with narrow blood sinusoids lined with endothelial cells and distinct phagocytic Kupffer cells. EAC-bearing mice revealed marked disorganization of their hepatic structure, dilated, and widening central vein, mostly hepatocytes are vacuolated and binucleated as well as aggregation of cellular infiltration was seen around the central vein (Figure 2B). Liver sections of mice treated with the low dose of Cis (0.125 mg/kg) shows disorganized hepatic strands, dilated and congested blood vessel, degenerated and vacuolated hepatocytes, and irregular blood sinusoids with distinct phagocytic Kupffer cells (Figure 2C). Liver sections of mice treated with the low dose of Cis (0.25 mg/kg) exhibited dilated central vein, most hepatocytes are markedly vacuolated with degenerated cytoplasm, aggregation of cellular infiltration, some hepatocytes show fainter or karyolytic nuclei (arrow); others appeared with condensed chromatin or pyknotic ones (Figure 2D). Liver sections of EAC-bearing mice treated with the low dose of Cis (0. 5 mg/kg) showed regular normal strands of hepatocytes with normal organized nuclei, congested, and dilated central vein, and regular blood sinusoids with normal Kupffer cells (Figure 2E).

Liver sections of EAC-bearing mice that treated with Cis (0.125 mg/Kg)/ EDTA (50 mg/Kg) showed an improvement of the hepatic architecture, most hepatocytes retain its stain affinity; their nuclei with normal distribution of chromatin, congested widening central vein and irregular blood sinusoids were slightly improved (Figure 2F). EAC-bearing mice that treated with Cis (0.25 mg/Kg)/EDTA (50 mg/Kg) showed an improvement of their hepatic architectures with regular radiating hepatic strands (Figure 2G). While liver sections of EAC-bearing mice treated with Cis (0.5 mg/kg)/EDTA (50 mg/kg) exhibits further improvement of the hepatic structure that was appeared as normal radiating hepatocytes with granular stained cytoplasm and centrically organized nuclei, normal central vein, and few hepatocytes are degenerated, regular distribution of sinusoids in-between hepatic cords with normal Kupffer cells (Figure 2H).

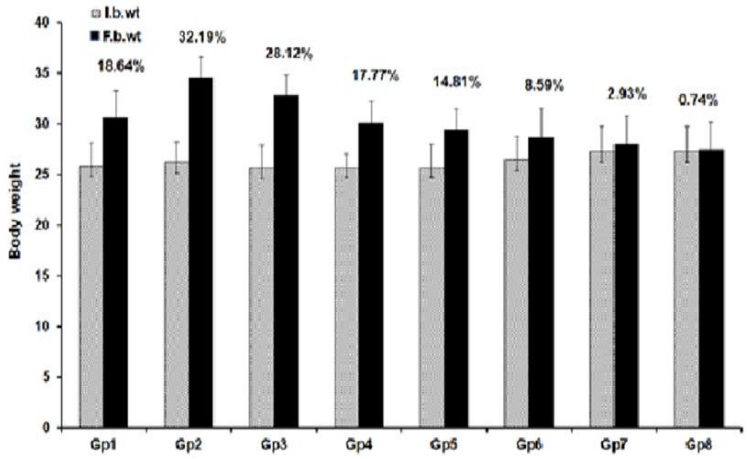

Fig. 1. Initial, final body weight, and the percentages of body weight changes in the different groups under the study. The values represented mean ± SD. Gp1: Control; Gp2: EAC-bearing mice alone; Gp3: EAC/Cis (0.125 mg/Kg); Gp4: EAC/Cis (0.250 mg/Kg); Gp5: EAC/Cis (0.5 mg/Kg); Gp6: EAC/Cis (0.125 mg/Kg)/EDTA (50 mg/Kg); Gp7: EAC/Cis (0.250 mg/Kg)/EDTA (50 mg/Kg); Gp8: EAC/Cis (0.5 mg/Kg) /EDTA (50 mg/Kg).

Table 1. The total tumor volume, tumor cells count and their live and dead numbers in different groups under the study.

Groups	T.T.V (ml/mouse)	T.T.C ($\times 10^6$/mouse)	T.L.C ($\times 10^6$/mouse)	T.D.C ($\times 10^6$/mouse)
Gp1	---	---	---	---
Gp2	7.2 ± 1.49[a]	1220 ± 264.3[a]	1220 ± 264.3[a]	1.0 ± 0.0[c]
Gp3	6.0 ± 0.91[a]	40.0 ± 3.74[b]	28.0 ± 2.83[b]	12.0 ± 2.16[a]
Gp4	4.2 ± 3.09[b]	38.0 ± 6.16[b]	24.0 ± 7.07[b]	14.0 ± 4.24[a]
Gp5	3.0 ± 0.82[b]	54.0 ± 29.1[b]	41± 26.6[b]	19.0 ± 3.37[a]
Gp6	2.8 ± 0.48[b]	32.0 ± 2.94[b]	17.0 ± 4.76[b]	15.0 ± 3.74[a]
Gp7	1.2 ± 0.48[b]	20.0 ± 3.4[b]	4.0 ± 2.5[b]	16.0 ± 1.63
Gp8	0.5 ± 0.48[b]	14.0 ± 4.69[b]	4.5 ± 4.04[b]	9.5 ± 1.0[b]

The values represented as mean ± SD. Gp1: Control; Gp2: EAC-bearing mice alone; Gp3: EAC/Cis (0.125 mg/Kg); Gp4: EAC/Cis (0.250 mg/Kg); Gp5: EAC/Cis (0.5 mg/Kg); Gp6: EAC/Cis (0.125 mg/Kg)/EDTA (50 mg/Kg); Gp7: EAC/Cis (0.250 mg/Kg)/EDTA (50 mg/Kg); Gp8: EAC/Cis (0.5 mg/Kg) /EDTA (50 mg/Kg); T.T.V: Total tumor volume; T.T.C: Total tumor count; T.L.C: Total live cells; T.D.C: Total dead cells. The low doses of Cis and EDTA were injected i.p for 6 consecutive days. Means that share letters are significantly different at P value < 0.05, while means that do not share letters are insignificant.

Table 2. Liver transaminases (ALT and AST) activity, creatinine, and urea levels in different groups under the study.

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Gp1	43.58 ± 4.4[f]	58.03 ± 3.9[f]	28.15 ± 1.5[f]	0.65 ± 0.05[c]
Gp2	160.75 ± 3.8[e]	228.3 ± 4.8 e	45.83 ± 2.2 a	1.44 ± 0.04[a]
Gp3	168 ± 17.33[a,b]	187 ± 18.48[abcd]	40.3 ± 5.33	1.25 ± 0.14[ab]
Gp4	154 ± 77.18[a]	170 ± 110.73[abc]	39.6 ± 12.92	1.05 ± 0.85[a]
Gp5	70.3 ± 2.39[a]	90.43 ± 3.5[a]	29.4 ± 2.4[b]	0.85 ± 0.03[b]
Gp6	123 ± 7.79[bc]	155 ± 63.27[abcd]	37.4 ± 17.29	0.95 ± 0.26[ab]
Gp7	93 ±2.38[c]	111± 4.99[bcd]	34.1 ± 12	0.90 ± 0.12[ab]
Gp8	52.15 ± 3.2[c]	65.43 ± 5.53[c]	21.28 ± 3.4[e]	0.64 ± 0.04[c]

The values represented as mean ± SD. Gp1: Control; Gp2: EAC-bearing mice alone; Gp3: EAC/Cis (0.125 mg/Kg); Gp4: EAC/Cis (0.250 mg/Kg); Gp5: EAC/Cis (0.5 mg/Kg); Gp6: EAC/Cis (0.125 mg/Kg)/EDTA (50 mg/Kg); Gp7: EAC/Cis (0.250 mg/Kg)/EDTA (50 mg/Kg); Gp8: EAC/Cis (0.5 mg/Kg) /EDTA (50 mg/Kg); ALT: alanine transferase; AST: aspartate transaminase. The low doses of Cis and EDTA were injected i.p for 6 consecutive days. Means that share letters are significantly different at P value < 0.05, while means that do not share letters are insignificant.

Table 3. Superoxide dismutase, catalase activities, glutathione reduced and malondialdehyde levels in different groups under the study.

Groups	SOD (U/mg tissue)	CAT (U/mg tissue)	GSH (µg/g tissue)	MDA (nmol/g tissue)
Gp1	21.44 ± 0.795 ^{a}	48.28 ± 1.7 ^{a}	18.2 ± 1.49 ^{a}	93.98 ± 2.39 ^{c}
Gp2	9.53 ± 1.84 ^{cd}	17.16 ± 1.92 ^{d}	10.82 ± 1.73 ^{ab}	157.42 ± 4.88 ^{ab}
Gp3	14.13 ± 0.4	20.6 ± 3.6 ^{c}	12.47 ± 0.46 ^{bc}	143.03 ± 3.5 ^{c}
Gp4	14.84 ± 0.38 ^{d}	25.2 ± 5.9 ^{cd}	12.20 ± 0.53 ^{c}	137.0 ± 4.9 ^{b}
Gp5	13.19 ± 1.31 ^{d}	29.54 ± 1.9 ^{e}	12.55 ± 2.3 ^{b}	127.68 ± 9.94 ^{a}
Gp6	17.27 ± 08 ^{c}	29.7 ± 3.5 ^{cd}	13.56 ± 0.39 ^{abc}	115.8 ± 3.4 ^{de}
Gp7	19.26 ± 0.9 ^{c}	36.1 ± 2.7 ^{cd}	14.9 ± 0.9 ^{ab}	109.61 ± 4.4 ^{d}
Gp8	17.22 ± 1.95 ^{bc}	35.82 ± 2.28 ^{e}	15.08 ± 2.298 ^{a}	115.11± 4.91 ^{ab}

The values represented mean ± SD. Gp1: Control; Gp2: EAC-bearing mice alone; Gp3: EAC/Cis (0.125 mg/Kg); Gp4: EAC/Cis (0.250 mg/Kg); Gp5: EAC/Cis (0.5 mg/Kg); Gp6: EAC/Cis (0.125 mg/Kg)/EDTA (50 mg/Kg); Gp7: EAC/Cis (0.250 mg/Kg)/EDTA (50 mg/Kg); Gp8: EAC/Cis (0.5 mg/Kg) /EDTA (50 mg/Kg); SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione reduced; MDA: malondialdehyde. The low doses of Cis and EDTA were injected i.p for 6 consecutive days. Means that share letters are significantly different at P value < 0.05, while means that do not share letters are insignificant.

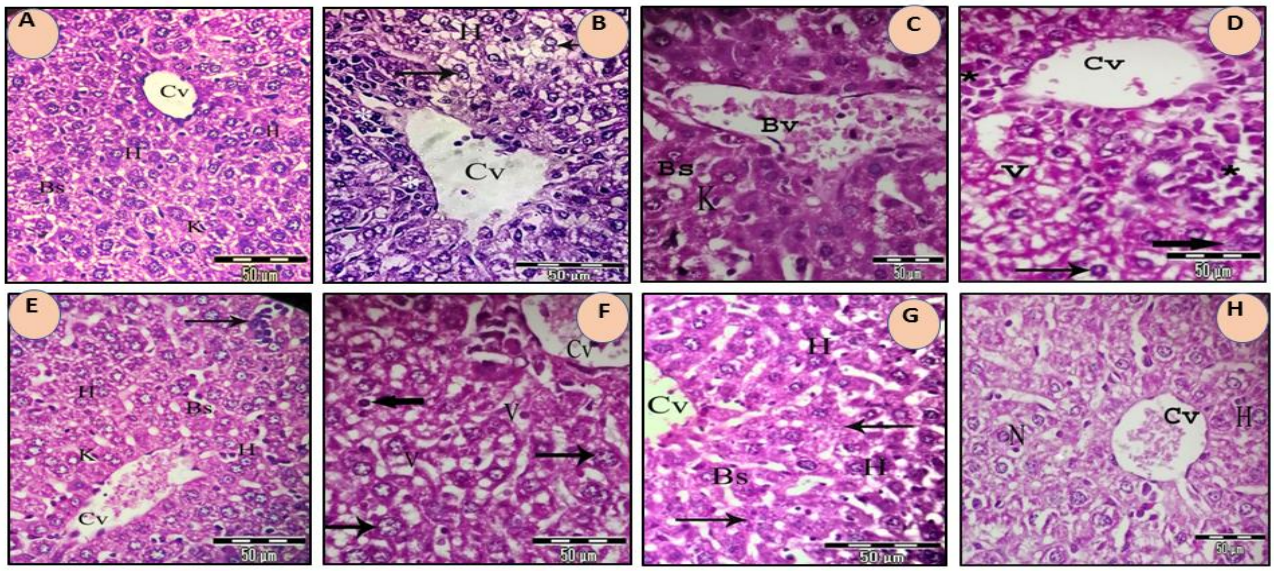

Fig. 2. A-H. Photomicrographic of the liver sections of different experimental animal groups stained with H&E (X 400). Liver section of control group (Gp1) showed the normal histological structure of hepatic lobule, central vein (Cv) and radiating polygonal hepatocytes (H), regular blood sinusoids (Bs) with normal phagocytic Kupffer cells (K) (Fig. 2A). The liver section of EAC-bearing mice (Gp2) showing marked disorganization of their hepatic structure, dilated, and widening central vein, mostly hepatocytes are vacuolated (H) and binucleated (arrows), also aggregation of cellular infiltration was observation (stars) (Fig. 2B). Liver sections of EAC-bearing mice that had treated with the low doses of Cis; Gp3: EAC/Cis (0.125 mg/Kg) (Fig. 2C), Gp4: EAC/Cis (0.250 mg/Kg) (Fig. 2D), Gp5: EAC/Cis (0.5 mg/Kg) (Fig. 2E) showing improvement in the hepatic lobule structures in dose dependent manner except some histopathological observations like degeneration of few hepatocyte (H) with pronounced nuclear changes like pyknotic nuclei (arrow) and karyolitic ones (thick arrow), congestion of some blood vessels (Bv) and blood sinusoids with distinct phagocytic Kupffer cells (K). Liver sections of EAC-bearing mice that had co-treated with the low doses of Cis and EDTA; Gp6: EAC/Cis (0.125 mg/Kg)/EDTA (50 mg/Kg) (Fig. 2F), Gp7: EAC/Cis (0.250 mg/Kg)/EDTA (50 mg/Kg) (Fig. 2G), Gp8: EAC/Cis (0.5 mg/Kg) /EDTA (50 mg/Kg) (Fig. 2H) showing more pronounced improvements in the hepatic architectures exhibit normal radiating hepatocytes (H) with granular stained cytoplasm (arrows) and centrically organized nuclei (N), normal central vein (Cv), and few number of hepatocytes are degenerated (thick arrow) and regular blood sinusoids are noticed (Fig. 2F, G and H).

4. Discussion

Chemotherapeutic drugs are used for the treatment of different malignancies, but the beneficial therapeutic effect is limited due to their adverse effects on the vital organs (Benzer et al., 2018). Cisplatin (Cis) is a chemotherapeutic drug that is effective against various types of cancers, but it has several undesirable side effects, so there was a great need in combination therapies of cis with other agents to reduce its toxicities (Aldossary, 2019; Arita et al., 2021). In this study, the direct effect of EDTA co-treatment on the efficacy of the low doses of Cis after inoculation of EAC cells has been investigated. This study showed that inoculation of EAC-cells led to an increase in the % of b.wt change, and this could be due to the proliferation of EAC-cells inside the peritoneal cavity of mice. This finding agreed with a previous study by El-Naggar et al. (2020), who reported that, was a significant increase in % b. wt change in EAC-bearing mice when compared to their normal control. The % of b.wt change post treatment of EAC-bearing mice with Cis (0.5 mg/Kg/6 days) was decreased. This finding agreed with previous studies, which used this model for drug screening (El-Naggar, 2019).

This finding agreed with previous studies showed the potential role of EDTA co-treatment on the efficacy of conventional chemotherapy (Duvillard et al., 2004; Feril et al., 2017; El-Naggar et al., 2019). Treatment of EAC-bearing mice with the low doses of Cis led to significant reduction in the total tumor volume, total tumor count, and total live tumor cells, however, these doses did not completely treat the EAC-bearing mice. These findings agree with the data obtained by El-Naggar et al. (2019). They reported that Cis reduces the percentage of T.V, T.C.C and T.L.C while increase the T.D.C. This could be due to the low doses of Cis is not enough to eliminate or stop the tumor cells completely. Co-treatment with different doses of EDTA increased the efficacy of the low doses of Cis as anticancer agents in EAC-bearing mice. In this study different doses of Cis and EDTA were used to optimize the treatment regimen that gives the highest effect of the low doses of Cis as anticancer in presence of EDTA. The results showed that among all the regimens, the treatment with 50 mg of EDTA and 0.5 mg of Cis/Kg for 6 days showed the optimal antitumor effect in EAC-bearing mice. This finding was supported by the decrease of the total volume, total tumor count, total live tumor cells, and increase in the total dead tumor cells. Previous study reported that EDTA alone is not a potential anticancer agent in EAC-bearing mice (El-Naggar et al., 2019; El-Naggar and El-Said, 2020). A previous study showed that the intraperitoneal injection with EDTA increased the antitumor effect of Cis (Hasinoff, 2006). In addition, it has been reported that EDTA might prevent iron from forming complex with DOX, which could prevent reactive oxygen species damaging (Duvillard et al., 2004). These results were in line with previous study reported that EDTA could enhance the antitumor efficacy of low dose of Cis in EAC-bearing mice by increasing the percentages of apoptotic tumor cells (El-Naggar et al., 2019; El-Naggar et al., 2022). Furthermore, previous study reported that the treatment with liposomes loaded with EDTA alone did not show any antitumor effects, however, liposomes loaded with EDTA and doxorubicin could significantly reduce drug toxicity without altering the antitumor activity (Song et al., 2014).

The results showed that liver transaminase, kidney function represented as urea and creatinine were significantly altered in EAC-bearing mice when compared to their values in naïve mice. ALT, AST, urea, creatinine, SOD, CAT, and GSH values were decreased post treatment of EAC-bearing mice with low doses of Cis in dose dependent manner. Co-treatment of EDTA with low doses of Cis significantly enhanced the functionality of liver and kidney organs and improved the antioxidant status represented by increase in the activity of SOD, CAT, and GSH and decreasing the level of MDA. Khalil et al. (2008) showed that treatment with EDTA altered antioxidant enzymes activity and

biochemical parameters in the serum. This finding agreed with the previous study, which showed that the treatment with Cis induces organs toxicity and increased the levels of liver enzymes, urea and creatinine (Ibrahim et al., 2019). In conclusion, the presence of EDTA at low concentrations increased the efficacy of the low dose of Cis as anticancer agent *in vitro* by using MCF-7 cells. The co-treatment with different doses of EDTA enhanced the efficacy of the low doses of Cis as anticancer agent in EAC-bearing mice.

Sections of liver tissues of EAC bearing mice showed marked disorganization of the hepatic architectures. These results agreed with Ahmed et al. (2017) who stated that liver section of EAC-bearing mice exhibited disorganized lobular architecture and these results agree with Attia et al. (2022) who reported that EAC cells can migrate from the peritoneal cavity and reach the liver causing liver injury. EAC-bearing mice that treated with Cis (0.125, 0.25 and 0.5 mg/kg) showed disorganization of their hepatic architectures, these results were parallel to that obtained by El-Naggar et al. (2015) who reported that hepatotoxicity and nephrotoxicity induced by Cis. The results revealed that liver sections of EAC-bearing mice that treated with Cis (0.125 mg/kg)/EDTA (50mg/kg), Cis (0.25 mg/kg)/EDTA (50 mg/kg) showed improvement of the hepatic structure represented by normal strands hepatocytes, few number of hepatocytes with degenerated cytoplasm. While liver sections of EAC-bearing mice that treated with Cis (0.5 mg/kg)/EDTA (50 mg/kg) showed more pronounced improvement of the hepatic architecture. These results were in parallel to El-Naggar et al. (2019) who stated that EDTA co-treatment enhanced the efficacy of the low dose of Cis at early and late time points of the treatment. In addition, EDTA co-treatment potentially ameliorated the Cis-induced side effects on liver and kidney functions. Collectively, Cis can induce deleterious effects even if it is given at low doses to EAC-mice; the presence of EDTA enhances Cis antitumor effects and improves the liver and kidney functions of EAC-bearing mice treated with Cis.

Conflicts of interest

There are no conflicts of interest to declare.

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