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# Effect of *Eremina desertorum* eggs on hepato-renal toxicity induced by cisplatin drug

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**ABSTRACT**

Cisplatin (Cis) is a chemotherapeutic drug that has been used in the treatment of various types of cancers. However, the drug showed various toxic side effects on vital organs. This study aims to investigate the hepato-renal protective effect of *Eremnia desertorum* eggs powder (EDEP) in Ehrlich Ascites Carcinoma (EAC) bearing mice treated with Cis. Five groups of female Swiss albino mice were allotted as follows: Group (Gp1) served as a negative control, Gp2-Gp5 had inoculated intraperitoneally (i.p) with $1\times10^6$ EAC cells/mouse. Then, Gp2 was left without treatment as a positive control (EAC-bearing mice). After 24 hours, Gp3, and Gp4 had injected i.p. with Cis (2 mg/kg) EDEP (500 mg/kg) daily for 7 consecutive days, respectively. Gp5 was injected with a combination of Cis/EDEP as in Gp3 and Gp4. All treated groups were sacrificed at day 12, and the percentage of the body weight changes (%b.wt), tumor indices, haematological and biochemical parameters, and antioxidant /oxidant biomarkers as well as histological alterations of liver and kidney tissues were investigated. The results showed that the % b.wt changes were increased in EAC-bearing mice and EAC-bearing mice that were treated with EDEP. While, the treatment of EAC-bearing mice with Cis decreased the % b.wt changes. The treatment of EAC-bearing mice with a combination of Cis/EDEP led to an improvement in AST, ALT, urea, creatinine, antioxidant enzyme activities, and ameliorated the histopathological toxicities in the liver and kidneys in EAC-bearing mice.

**Keywords:**

Antioxidants, Cisplatin, EAC-cells, Eggs, *Eremina desertorum*, Hepato-renal protective, Powder

## 1. Introduction

Cancer is one of the leading causes of death around the world; it is characterized with the uncontrolled growth of cells. (Proksch et al., 2002). Cancer is treated with surgery, radiation, chemotherapy, hormone therapy, biological therapy and targeted therapy. Chemotherapy remains the dominant weapon for the treatment of cancer. Chemotherapy kills not only the tumor cells but also normal cells (Garcia-Fernandez et al., 2002). Chemotherapy drugs are cytotoxic, non-specific and associated with wide range of side-effects. Chemotherapy drugs can also cause long lasting side effects such as damage to lung tissue, heart problems, infertility, kidney problems, nerve damage, risk of a second cancer (Nastoupil et al., 2012) .

Cisplatin (Cis) is used in the treatment of various types of cancers such as ovarian, lung, head and neck, testicular and bladder. Treatment with Cis is associated with various toxic side effects (Singh et al., 2018). Furthermore, low doses of Cis which are probably due to cumulative effect in the liver cause massive hepatic toxicity, including dissolution of hepatic cords, focal inflammatory lesions and necrosis (Singh et al., 2015). On the other hand, the long-term effect of Cis on renal function is permanent reduction in

glomerular filtration rate (Arany and Safirstein. 2003). The mechanisms of Cis nephrotoxicity involve oxidative stress, apoptosis, inflammation, and fibrogenesis. High concentrations of Cis induce necrosis in proximal tubule cells, whereas lower concentrations induce apoptosis (Lieberthal et al., 1996). Natural products have a long history of use in the service of mankind for the prophylaxis and treatment of several diseases. It has been reported that one-third of cancer patients use some form of complementary and other alternative medicines. Recently considerable attention has been focused on using natural products for chemoprevention due to their considerable safety and efficacy in the treatment (Girdhani et al., 2005).

Molluscs are currently used for a range of therapeutic applications, with purified or synthesized bioactive compounds developed as pharmaceuticals agents (Mayer et al., 2010). Class gastropoda form a major part of the phylum Mollusca. Gastropods are more commonly known as snails and slugs. Some studies reported the medicinal properties and bioactive potential of snail's extracts as antioxidant, antibacterial, antifungal, analgesic, anti-inflammatory and anticancer potential (Adikwu, 2007; Zhang and Cunshe, 2013; Harti et al., 2016; Cilia and Fratini, 2018) .

The desert snails *Eremina desertorum* live in sandy deserts and feed on shrubs (Ali, 2017). It is one of the common desert species that occurs in many different locations along the Mediterranean region, between Alexandria till the border of Egypt with Libya (Ali et al., 2016). Ibrahim et al. (2022) reported that *E. desertorum* mucin could be used as a potential hepatoprotective, antioxidant and anti-inflammatory agent for hepatic disorders against CCl4 induced hepatotoxicity. *E. desertorum* mucin was also found to have antioxidative and anti-cancer activity against two types of tumor cells; CACO-2 and HepG2 cells (Atta et al., 2021). El-Zawawy and Mona. (2021) reported that mucus extract of *E. desertorum* gave higher inhibitory activity against resistant strains related to burn wound infections. Also, it revealed a significant anti-inflammatory activity. So, the current study conducted to evaluate E. desertorum eggs hepatic-renal protective effects on toxicity induced by Cis in EAC-bearing mice.

## 2. Materials and methods

### Chemicals

Cisplatin (Cis) was purchased from Sigma-Aldrich (St. Louis, Mo., USA), and diluted by 0.9% normal saline and adjusted to 2 mg/kg b.wt in 200 µL. Absolute ethanol and formalin reagents were purchased from commercial company (Al-Gomhorea, Tanta, Egypt). Aspartate amino transferees (AST), alanine amino transferees (ALT), urea and creatinine, super oxide dismutase (SOD), Catalase (CAT) and malondialdehyde (MDA) kits were purchased from Biodiagnostic Company, Egypt.

### Snail collection and eggs powder preparation

Snails (*E. desertorum*) were collected from the desert in the south of Alamein, Egypt during September 2022 and transported to Zoology Department Faculty of Science, Tanta University. The samples were housed in glass boxes with 10-15 mm sand at the bottom, each with 20-25 snails. To keep the boxes damp, they were sprayed with water daily. The temperature ranged between 20 to 22 ºC and the snails were fed on fresh cucumber. The snails` eggs were collected from sand, washed by dechlorinated tap water, kept in a sterile tube, homogenized and lyophilized to obtain *E. desertorum* eggs powder (EDEP) which preserved at (- 80 ºC) until use.

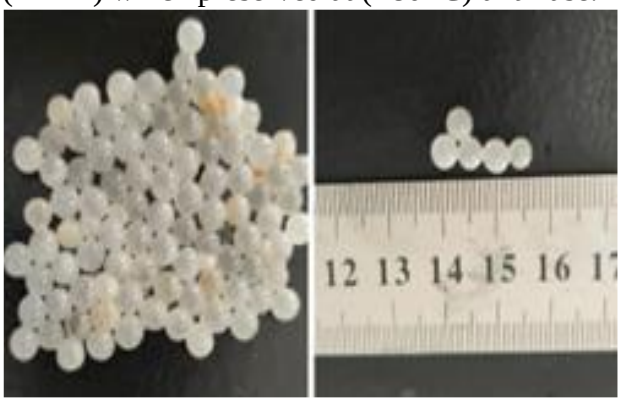


**Fig. 1.** Photomicrograph shows *E. desertorum* eggs. *E. desertorum* snails were collected, housed and the eggs were collected from sand, washed by dechlorinated tap water, then photographed.

### Experimental mice

Seventy-four female Swiss albino mice (20 ± 2 g) were obtained from animal house colony Egyptian Vaccine Company, Giza, Egypt. Mice were kept for a week before starting the experiment for adaptation under convenient

temperature (22 ± 1ºC), relative humidity (55 ± 5%), and light- dark (day/night) cycle was achieved. Mice were given drinking tap water and normal pelleted animal food ad *libitium* according to the ethical guidelines (IACUC-SCI-TU-0249) approved by the animal care and use committee, Faculty of Science, Tanta University

**Determination of median lethal dose of EDEP**

A total number of 24 female mice were divided in to six groups (n = 4). Mice from each group were injected intraperitoneal (i.p) with a single dose of 0.5. 1, 2, 3, 4 and 5 g /Kg b.wt of EDEP. Mice were noticed for 24 h to assess the acute toxicity of EDEP. Median lethal dose ($LD_{50}$) value was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

**EAC cells expansion and inoculation**

Tumor cells were obtained from Cairo University, Egypt (National Cancer Institute). Each mouse was injected with $1 \times 10^6$ EAC-cells i.p. For tumor progression, EAC-injected mice were monitored daily for abdominal distention, distress and illness.

**Experimental design**

Mice were divided into 5 groups (n=10) as follows: Group 1 (Gp1) was kept as negative control. Gp2, Gp3, Gp4 and Gp5 were inoculated with 1 x $10^6$ EAC-cells /mouse i.p. Gp2 was left untreated and served as positive control. Post 24 h of inoculation, Gp3 and Gp4 were injected with Cis (2 mg/Kg) and EDEP (100 mg/Kg) i.p. for 7 consecutive days, respectively. Gp5 was injected with a combination of Cis/EDEP as in Gp3 and Gp4. By the end of the experiment at day 12, animals were sacrificed. Via orbital plexus, blood samples were collected for determination of biochemical and hematological parameters.

**Determination of % b.wt change, absolute and relative organs weight**

At the beginning (Ib) and end of experiment (Fb), animals were weighed and percentage of body weight changes (% B.wt) were calculated according to the equation;

B. wt (%) = {(Fb-Ib)/Ib}x100.

**Determination of tumor profile**

The ascetic fluid was collected from the peritoneal cavity of untreated and treated EAC-bearing mice groups. The tumor volume was measured using graduated centrifuge tube. EAC cells have been suspended in sterile isotonic saline, and the total tumor cell number was counted using the hemocytometer (Bothara et al., 2015).

**Determination of hematological and biochemical parameters**

Complete blood parameters were evaluated using blood counter (Dirui BCC 3600, MA, USA. Serum aminotransferases (ALT and AST) activities, urea, and creatinine levels were determined by colorimetric methods according to the Bio-diagnostic instruction. SOD and CAT were assayed according to the method of Christine and Joseph (2010). MDA level was determined according to the method of Prima (2018).

**Histological examination**

Small pieces of liver and kidney from different experimental groups were immediately fixed in 10% neutral buffered formalin for 24 h. The tissue samples were dehydrated in ascending concentrations of ethyl alcohol, cleared by xylene, and embedded in paraffin. Sections of 5-mm-thick were mounted and stained with hematoxylin and eosin (H&E) according to the Bancroft and Gamble protocol (2008). All slides were microscopically examined for histopathological alterations by light microscope (Olympus, Model: CX21FS2).

**Statistical analysis**

All data are the means of 3 replicates. The data were expressed as mean ± SD. Comparison between groups was carried out using one-way ANOVA. If there a significant difference between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests p values <0.05 was considered to be statistically significant.

## 3. Results

Median lethal dose ($LD_{50}$) of EDEP on mice

Different doses of EDEP ranging between 0.5– 5 g/Kg were injected in different groups of mice, after 24 hours, there was no mortality recorded in all groups under experiment. (Data not shown)

**Effect of EDEP on the percentage of body weight changes**

EAC-bearing mice showed a significant increase in the % b.wt change, when compared to the

control group ($p < 0.05$). EAC-bearing mice that had treated with Cis showed a significant decrease in the % b.wt change when compared to the EAC-bearing mice alone ($p < 0.05$). EAC-bearing mice that had treated with EDEP also showed significant increase in the % b.wt change when compared to the control ($p < 0.05$), with no significant difference when compared to the EAC-bearing mice ($p \geq 0.05$). EAC-bearing mice that had treated with a combination of Cis/EDEP showed significant decrease in the % b.wt change when compared to the EAC-bearing mice ($p < 0.05$) with no significant difference when compared to Cis treated EAC-bearing mice ( $p \geq 0.05$) (Fig. 2).

**Effect of EDEP on the tumor profile in EAC-bearing mice**

As compared to the EAC-bearing mice, treatment with Cis showed a significant decrease ($p < 0.05$) in the total tumor volume (0.5 ± 0.02 ml/ mouse) Treatment with EDEP showed no significant difference in the total tumor volume ($p \geq 0.05$). The total count of EAC cells in EAC-bearing mice was $350\pm40\times10^6$ / mouse. Treatment with Cis showed a significant decrease ($p < 0.05$) in the number of EAC cells ($36\pm3\times10^6$/ mouse) when compared to untreated EAC-bearing mice group. Treatment with EDEP showed no significant difference in the number of EAC cells ($320\pm35\times10^6$/ mouse) when compared to EAC group ($p \geq 0.05$) (Fig. 3).

**Effect of the treatment with Cis and/or EDEP on hematological parameters of EAC-bearing mice**

As compared to control group, EAC-bearing mice treated with Cis and EAC-bearing mice treated with combination of EDEP and Cis didn't show significant alterations in the total red blood cells count (R.B.Cs), hemoglobin (Hb) level, hematocrit (Hct) % and the platelets count ($p \geq 0.05$). EAC-bearing mice and EAC-bearing mice treated with EDEP showed significant decrease in the total red blood cells count (R.B.Cs), hemoglobin (Hb) level, hematocrit (Hct) %,and the platelets count ($p < 0.05$) ( Table 1).

The total white blood cells (W.B.Cs) count and their differential were significantly increased in EAC-bearing mice ($p < 0.05$). EAC-bearing mice that had treated with Cis showed significant decrease in the total W.B.Cs when compared to the EAC-bearing mice group ( $p < 0.05$). EAC-bearing mice that had treated with EDEP showed partial alteration when compared to the EAC-bearing mice group ($p \geq 0.05$) (Table 2).

**Effect of the treatment with Cis and/or EDEP on liver and kidney functions of EAC-bearing mice**

EAC-bearing mice showed significant increase in the activity of liver transaminases (ALT, AST) when compared to the control group ($p \leq 0.05$). EAC-bearing mice that had been treated with Cis showed significant decrease in the activity of liver transaminases when compared to EAC-bearing mice group ($p \leq 0.05$). EAC-bearing mice that had been treated with the EDEP showed a significant difference in the activity of liver transaminases when compared to the EAC-bearing mice group ($p \leq 0.05$). EAC-bearing mice that had treated with a combination of Cis and EDEP showed significant decrease in activity of liver transaminases when compared to EAC-bearing mice group ($p \leq 0.05$) (Fig. 4).

The levels of urea and creatinine in EAC-bearing mice were significantly increased when compared to control group ($p \leq 0.05$). EAC-bearing mice that had treated with cis showed significantly decrease in levels of urea and creatinine when compared to EAC-bearing mice group ($p \leq 0.05$). EAC-bearing mice that had been treated with EDEP showed a significant decrease in the levels of kidney biomarkers when compared to EAC-bearing mice group ($p \leq 0.05$). EAC-bearing mice that had been treated with Cis and EDEP showed significant decrease in the levels of urea and creatinine when compared to EAC-bearing mice group ($p \leq 0.05$) (Fig. 5).

**Effect of the treatment with EDEP on antioxidants/oxidant biomarkers**

The results showed that there were significant differences in the hepatic oxidant/ antioxidant biomarkers, superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) between the control group and the EAC-bearing mice groups ($p \leq 0.05$). EAC-bearing mice showed significant decrease in SOD and CAT activities with significant increase in the MDA level when compared to the control group ($p \leq 0.05$). EAC-bearing mice that had been treated with Cis showed significant increase in the SOD and CAT activities with significant decrease in

MDA level when compared to the EAC-bearing mice ($p \leq 0.05$). EAC-bearing mice that had been treated with EDEP showed a significant increase in SOD and CAT activities with significant decrease in the level of MDA when compared to EAC-bearing mice group ($p \leq 0.05$). EAC-bearing mice that had treated with a combination of Cis and EDEP showed significant increase in SOD and CAT activities with significant decrease in MDA level when compared to EAC-bearing mice group ($p \leq 0.05$) (Table 3).

**Histopathological changes in liver tissue after treatment with Cis and/or EDEP in EAC-bearing mice**

Examination of H&E-stained sections of liver from control mice (Gp1) showed normal hepatocyte architecture with hepatic lobulation. Hepatic strands alternated with narrow blood sinusoids lined by an endothelial cell layer and Kupffer cells. Hepatocytes with centrally located nuclei and normally distributed chromatin (Fig. 6A). Liver section of EAC-bearing mice exhibits marked disorganization of the hepatic structure, severe congested central veins, mostly hepatocytes are degenerated with vacuolated cytoplasm, dilated and widening blood sinusoids with distinct Kupffer cells and severe mononuclear infiltration was noticed (Fig. 6B). Liver section of mice that had treated with EAC/Cis showing improvement of the hepatic architecture represented by regular central vein, mostly hepatocytes are normal, few numbers are degenerated with vacuolated cytoplasm, others with abnormal nuclei like megakaryocytic ones, deteriorated and narrow blood sinusoids with normal Kupffer cells (Fig. 6C). Liver section of mice that had treated with EDEP extract showing normal like structure of hepatic architecture, normal radiating hepatocytes with normal nuclei, enlarged central vein, mostly hepatocytes are normal, few numbers are binucleated, widening blood sinusoids with distinct phagocytic Kupffer cells were noticed (Fig. 6D). Liver section of mice that had treated with EDEP/Cis exhibits improvement of the hepatic architecture, normal radiating hepatocytes with normal nuclei, enlarged central vein, few number of hepatocytes with vacuolated cytoplasm, others with pyknotic nuclei, regular blood sinusoids with normal Kupffer cells were noticed. (Fig. 6E)

**Histopathological changes in kidney tissue after treatment with Cis and/or EDEP in EAC-bearing mice**

Kidney Histopathology. Light microscopic examination of the renal cortex of mice from the control group (Gp1) displayed a typical (normal) appearance. Bowman's capsule surrounds the glomerulus, which is bordered by two layers of epithelium. The glomeruli are round and oval in shape, the proximal convoluted tubules were lined with the simple cuboidal or columnar epithelium, its cells had an acidophilic cytoplasm, and the apex possesses abundant microvilli which formed a brush border. The distal convoluted tubules were lined with the simple cuboidal epithelium (Fig. 7A). The kidney tissues of EAC-bearing mice showed disorganization of the glomeruli with atrophy of its bowman's space, mostly renal tubules are disorganized and damaged, lost their characteristic appearance and atrophy of its lining epithelia, other tubules with cloudy swelling, as well as inter-tubular hemorrhage and aggregation of inflammatory cells were noticed (Fig. 7B). While the kidney sections of mice that had treated with EAC/Cis revealed few numbers of the glomeruli are normal, others are disorganized with narrow bowman's space, few numbers of the renal tubules are normal, mostly ones are damaged and lost their lining epithelia (Fig. 7C). In the treated group with EDEP extract, a renal profile is nearly restoring its normal anatomical appearance, normal glomeruli with regular bowman's space, mostly no of the renal tubules are normal, others are distended, and their contents are intermixed with each other (Fig. 7D).

On examination of the kidney of mice treated with EDEP/ Cis improvement in the renal architecture represented by normal glomeruli with regular bowman's space, mostly renal tubules are normal, few number are damaged with necrotic and disrupted lining epithelia (Fig. 7E).

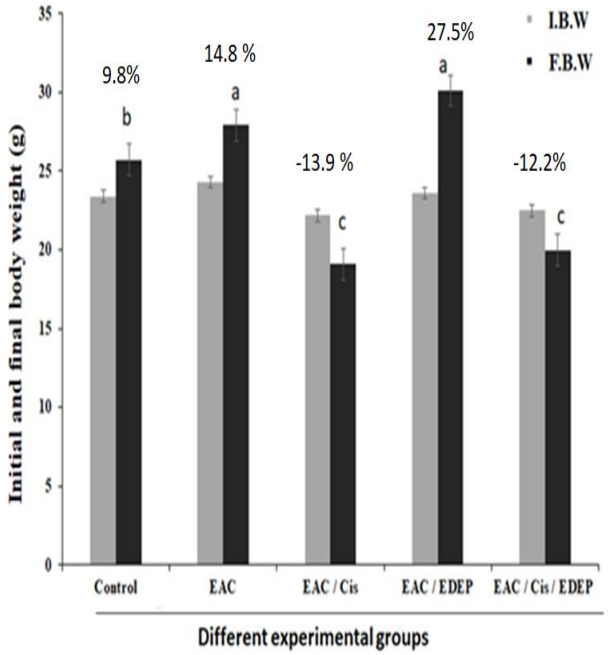


**Fig. 2.** The initial and final body weight in different experimental groups

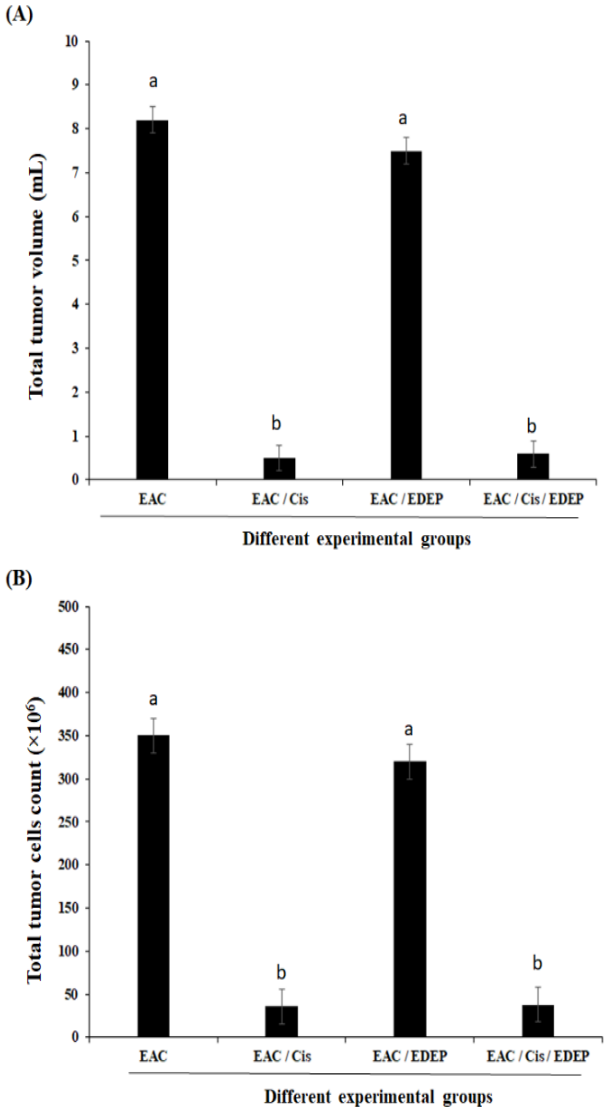


**Fig.3.** Total tumor volume and total cell count in EAC- bearing mice

**Table 1.** The haematological parameters in different groups of mice

| Groups | R.B.Cs (×10$^6$/ul) | Hb (g/dl) | Hct% | Platelets (×10$^3$/ul) |
|---|---|---|---|---|
| Ctrl | 9.7 ± 0.4$^a$ | 15.34 ± 0.7$^a$ | 44.2 ± 2.4$^a$ | 649.6 ± 217.6$^a$ |
| EAC-alone | 7.8 ± 0.5$^b$ | 13.06 ± 0.8$^b$ | 38.6 ± 2.3$^b$ | 585.4 ±796.2$^b$ |
| EAC/Cis | 9.0 ± 2.0$^a$ | 14.88 ± 3.2$^a$ | 42.9 ± 12.7$^a$ | 628.4 ± 153.9$^a$ |
| EAC/EDEP | 8.1 ± 0.4$^b$ | 13.16 ± 1.1$^b$ | 39.92 ± 3.06$^b$ | 572.2 ± 111.4$^b$ |
| EAC/Cis/EDEP | 9.3 ± 1.2$^a$ | 14.70 ± 1.9$^a$ | 43.5 ± 6.4$^a$ | 633.4 ± 155.7$^a$ |

Data expressed as mean ± SD; Ctrl: control; EAC: EAC-bearing mice; Cis: Cisplatin; EDEP: *E. desertorum* eggs extract; R.B.Cs: Red blood cells; Hb: Hemoglobin; Hct: Hematocrit. p value ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

**Table 2.** Total white blood cells count and the absolute of differential leucocytes of different groups.

| Groups | W.B.Cs (×10$^3$/ul) | Monocytes (×10$^3$/ul) | Neutrophils (×10$^3$/ul) | Lymphocytes (×10$^3$/ul) |
|---|---|---|---|---|
| Ctrl | 5.8 ± 1.2$^b$ | 0.1 ± 0.09$^a$ | 2.0 ± 0.4$^b$ | 3.6 ± 0.8$^b$ |
| EAC-alone | 29.5 ± 12.1$^a$ | 2.1 ± 0.8$^a$ | 11.2 ± 7.1$^a$ | 16.2 ± 3.9$^a$ |
| EAC/Cis | 10.7 ± 3.8$^b$ | 0.1 ± 0.03$^a$ | 4.6 ± 0.6$^b$ | 6.3 ± 2.2$^b$ |
| EAC/EDEP | 25.5 ± 9.0$^a$ | 2.2 ± 0.9$^a$ | 9.4 ± 1.9$^a$ | 13.8 ± 4.1$^a$ |
| EAC/Cis/EDEP | 15.4 ± 6.3$^{a,b}$ | 0.9 ± 0.6$^a$ | 4.3 ± 1.2$^{a,b}$ | 10.1 ± 2.8$^{a,b}$ |

Data expressed as mean ± SD; Ctrl: control; EAC: EAC-bearing mice; Cis: Cisplatin; EDEP: *E. desertorum* eggs extract; W.B.Cs: White blood cells. p value ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

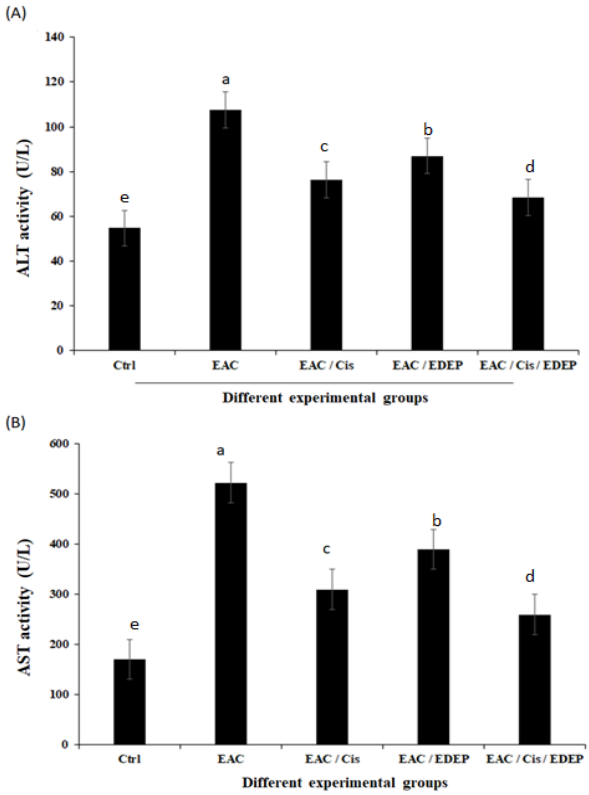


**Fig. 4.** ALT and AST activities in different experimental groups.

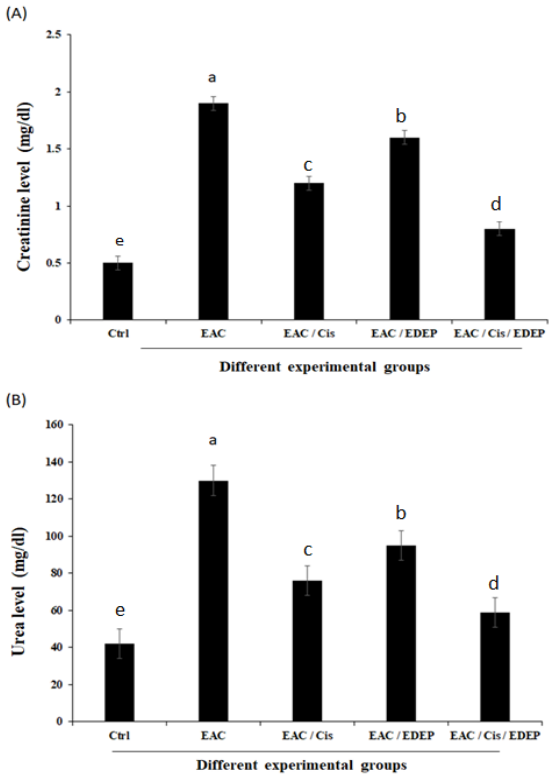


**Fig. 5.** Creatinine and urea levels of different experimental groups.

**Table 3.** Hepatic SOD, CAT activity and MDA level in the different groups.

| Groups | SOD (U/mg tissue) | CAT (U/g tissue) | MDA (nmol/g tissue) |
|---|---|---|---|
| Ctrl | 5.1 ± 0.2 a | 76.6 ± 2.7 a | 30.8 ± 1.4 e |
| EAC-alone | 1.5 ± 0.1 e | 32.3 ± 2.3 e | 90.1 ± 3.2 a |
| EAC/Cis | 3.0 ± 0.07 c | 56.7 ± 3.1 c | 52.3 ± 1.9 c |
| EAC/EDEP | 2.4 ± 0.1 d | 43.1 ± 1.4 d | 68.8 ± 3.3 b |
| EAC/Cis/EDEP | 3.8 ± 0.1 b | 64.9 ± 1.8 b | 41.9 ± 1.6 d |

Data expressed as mean ± SD; Ctrl: control; EAC: EAC-bearing mice; Cis: Cisplatin; EDEP: *E. desertorum* eggs extract; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde. p value ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

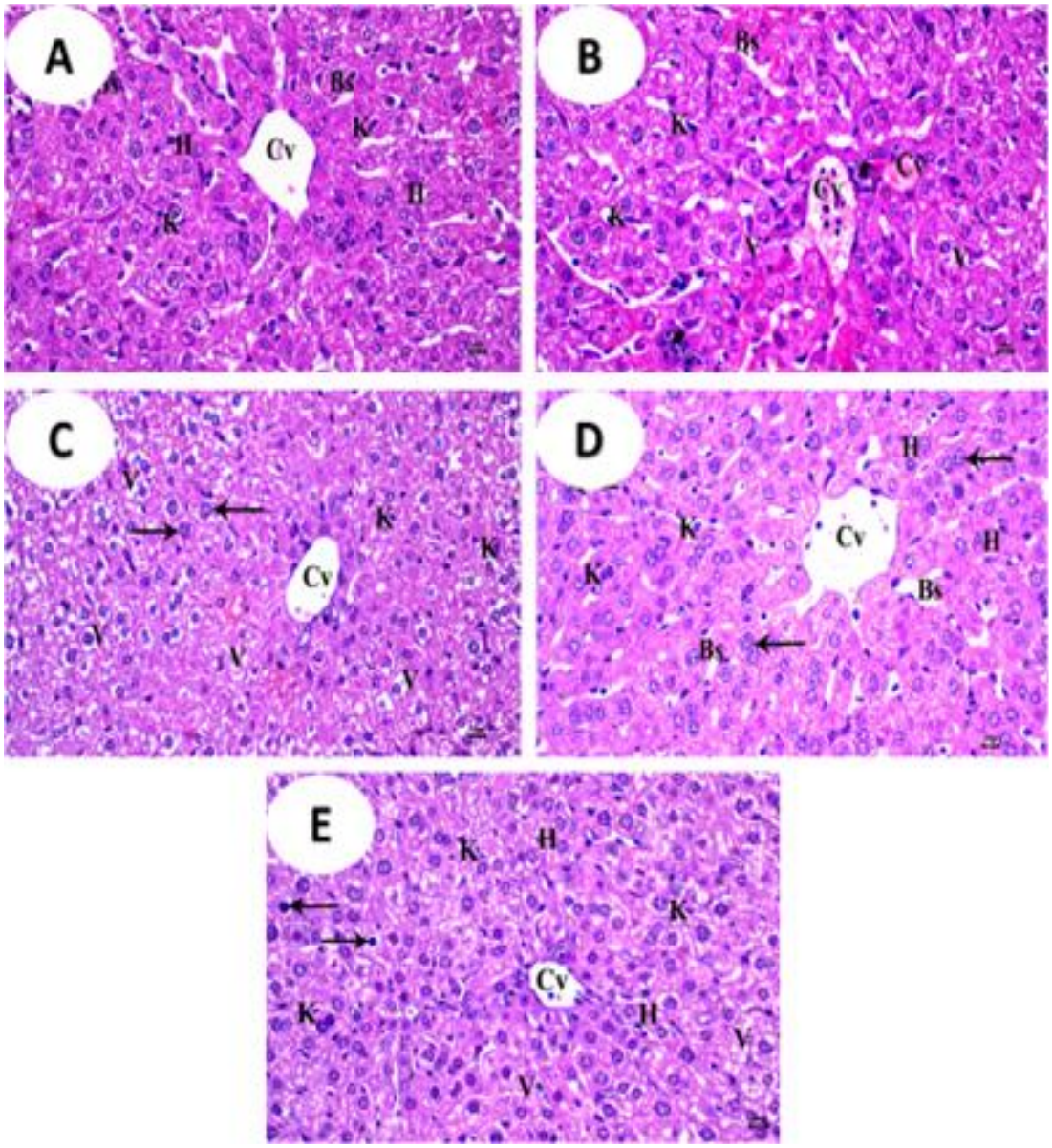


**Fig 6**. Photomicrograph of liver sections of treated groups. Liver section of control mice showing central vein (Cv), normal radiating polygonal hepatic cells (H), regular blood sinusoids (Bs), which contain normal Kupffer cells (K). (A). Liver section of EAC-bearing mice exhibits disorganization of hepatic structure, severe congested central veins (Cv), mostly hepatocytes are degenerated with vacuolated cytoplasm (V), dilated and widening blood sinusoids with distinct Kupffer cells (K) and severe mononuclear infiltration was noticed (stars). (B) Liver section of EAC/Cis mice showing improved hepatic architecture like regular central vein (Cv), mostly hepatocytes are normal, few numbers are degenerated (V), others with megakaryocytic nuclei (arrows), deteriorated blood sinusoids with normal Kupffer cells (K). (C). Liver section of EAC/EDEP group showing normal like structure of hepatic architecture, normal radiating hepatocytes (H) with normal nuclei, enlarged central vein (Cv), few numbers of hepatocytes are binucleated (arrows), widening blood sinusoids (Bs) with distinct phagocytic Kupffer cells were noticed (K). (D). Liver section of EAC/Cis/EDEP group exhibits improvement of the hepatic structure, normal radiating hepatocytes (H) with normal nuclei, enlarged central vein (Cv), few numbers with vacuolated cytoplasm (V), others with pyknotic nuclei (arrows), regular blood sinusoids with normal Kupffer cells were noticed (K). (E). (H&E stain).

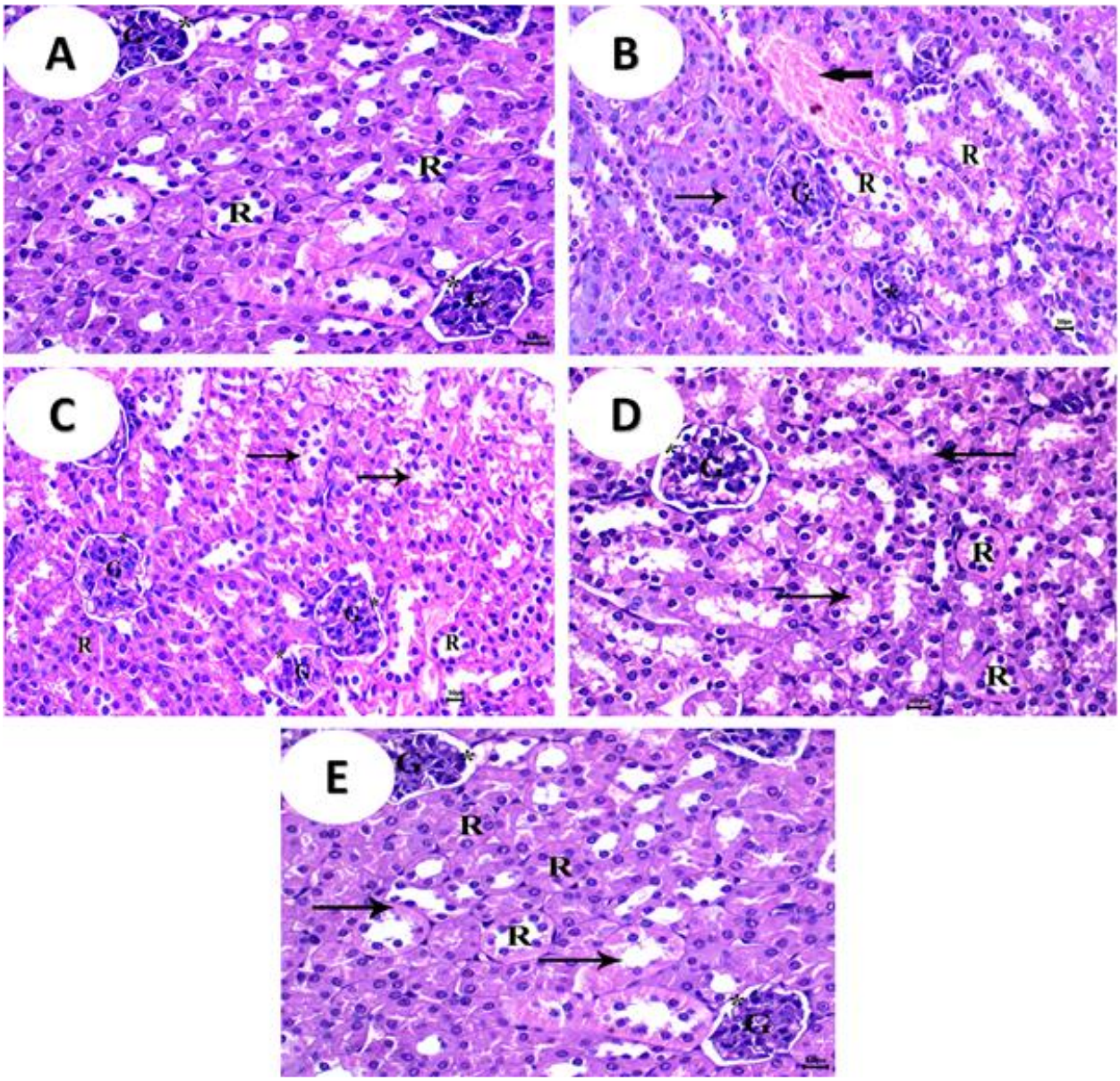


**Fig 7.** Photomicrograph of kidney sections of treated groups. Kidney section of control mice showing normal architecture of renal glomeruli (G) with normal Bowman's space and renal tubules (R). (A). Kidney section of EAC-bearing mice exhibits disorganization of the glomeruli (G) with atrophy of its Bowman's space, mostly renal tubules are disorganized (R), lost their characteristic appearance and atrophy of its lining epithelia, other tubules with cloudy swelling (arrows), as well as inter-tubular hemorrhage (thick arrow) and aggregation of inflammatory cells were noticed (star). (B). Kidney section of EAC/Cis mice reveals few numbers of the glomeruli (G) are normal, others are disorganized with narrow Bowman's space (*), few numbers of the renal tubules are normal (R), mostly ones are damaged and lost their lining epithelia (arrows). (C). Kidney section of EAC/EDEP mice showing normal like structure of the renal structure; represented by normal glomeruli (G) with regular bowman's space (*), mostly no of the renal tubules are normal (R), others are distended, and their contents are intermixed with each other (arrows). (D). Kidney section of EAC/Cis/EDEP group showing improvement in the renal architecture represented by organized glomeruli (G) with regular Bowman's space (*), mostly renal tubules are normal, few number are damaged with disrupted lining epithelia (arrows) (E). (H&E stain).

## 4. Discussion

The current study showed that the powder of *E. desertorum* eggs was safe and showed no toxicities up to 5000 mg/ Kg. Till now, there are no previous studies reporting $LD_{50}$ of EDEP. EAC-bearing mice showed an increase in the % b. wt. change when compared to the control group, this could be due to proliferation of tumor cells and accumulation of ascetic fluid in the peritoneal cavity, and this may cause hyper permeability of micro- blood vessels to lead to an enlargement of the abdomen. These findings were consistent with the previous study by Saad et al. (2017) and El-Naggar et al. (2023).

Treatment of EAC-bearing mice with Cis led to a decrease in % b. wt. change. This finding is due to the prohibiting effect of Cis and consistent with previous studies by Dasari and Tchounwou, (2014) and El-Naggar et al. (2023). EAC-bearing mice that were treated with EDEP showed no significant difference in % b. wt. change when compared to the EAC-bearing mice. This finding may explain that the EDEP has no antitumor effect on EAC cells. EAC-bearing mice that had been treated with a combination of Cis and EDEP showed a significant decrease in %b. wt. change when compared to EAC-bearing mice alone with no significant difference to EAC-bearing mice that had been treated with Cis. This finding postulated that EDEP does not affect the efficacy of Cis treatment. EAC-bearing mice showed significant increase in the total tumor volume and the total cells count because of the accumulation of the ascetic fluid in the peritoneal cavity and the proliferation of tumor cells. These findings are consistent with Ozaslan et al. (2011). Treatment EAC-bearing mice with Cis led to significant decrease in tumor volume and total cells count when compared to EAC-bearing mice group and this is because the antitumor effect of Cis, this result agrees with El-Naggar et al (2023) and Dasari and Tchounwou, (2014). Treatment EAC- bearing mice with EDEP showed no difference when compared to EAC-bearing mice in the total tumor volume nor the total cells count, and this proves that the EDEP has no antitumor effect. Based on search there are no previous studies in vitro nor in vivo reporting the anti-tumor effect of EDEP. However, El-Zawawy and Mona. (2021) reported that mucus extract of *E. desertorum* lacks cytotoxicity effect. Another study reported that *E. desertorum* mucin was found to have anti-cancer activity against two types of tumor cells; CACO-2 and HepG2 cells (Atta et al., 2021). A recent study on a similar species reported that the anti-tumor effect of *Helix aspersa* eggs on colon cancer cells (Caco-2) (Matusiewicz et al., 2022). Treatment EAC-bearing mice with combination of Cis/EDEP showed significant decrease in the total tumor volume and the total cells count as same as EAC-bearing mice that had treated with Cis only which means that EDEP did not affect the efficacy of Cis .

EAC-bearing mice and EAC-bearing mice treated with EDEP showed significant decrease in the total RBCs count, Hct value, Hb level and platelets count when compared to the other groups, this could be due to the suppressive effect of EAC toxin on the bone marrow and erythropoiesis. A previous study reported that hematological parameters of EAC-bearing mice showed reduction in total RBCs count, Hct value, Hb level and platelets count (Hashem et al., 2020). Treatment with Cis led to improvement in total RBCs count, Hct value, Hb level and platelets count close to the control group. Treatment with EDEP led to no change in total RBCs count, Hct value, Hb level and platelets count when compared to EAC-bearing mice alone. Treatment with a combination of Cis/EDEP showed improvement in the total RBCs count, Hct value, Hb level and platelets count as same as EAC-bearing mice that was treated with Cis only which indicates that EDEP did not affect Cis efficacy. The data reported that EAC-bearing mice showed an increase in the total W.B.Cs count when compared to the control group. This could be due to the inflammatory response and stress of EAC cells proliferation. In a previous study showed significant elevation in total W.B.Cs count in EAC-bearing mice (Hashem et al., 2020). EAC-bearing mice treated with Cis significant decrease in W.B.Cs count when compared to EAC-bearing mice and this could be due to the antitumor effect. This agreed with data obtained by Hashem et al. (2020). Treatment with EDEP led to a substantial difference in the total

W.B.Cs count in EAC-bearing mice as compared to EAC-bearing mice or EAC-bearing mice treated with Cis.

In current study, ALT and AST activities significantly increased in the EAC-bearing mice when compared to control group, this is due to the hepatic damage (Khalifa et al., 2011). This finding is consistent with data obtained by Gowda et al. (2022). EAC-bearing mice treated with Cis led to decrease in ALT and AST activities when compared to EAC-bearing mice and this finding is consistent with a previous study by Salama et al. (2022). Treatment of EAC-bearing mice with EDEP showed decrease in liver transaminases activities when compared to EAC-bearing mice alone which means that EDEP has a protective effect on liver. Based on search, till now no previous studies reported the ameliorative effect of EDEP on liver and kidney. A previous study by Ibrahim et al. (2022) declared that *E. desertorum* mucin could be used as a potential hepatoprotective agent for hepatic disorders against $CCl_4$. EAC-bearing mice treated with a combination of Cis and EDEP showed significant decrease in ALT and AST activities, and this could be due to the synergistic effect between Cis and EDEP. EAC-bearing mice showed significant increase in kidney biomarkers levels when compared to control group this could be due to muscle necrosis catabolic effect of the tumor and the elevation in urea production and this is consistent with Salama et al. (2022). Treatment of EAC-bearing mice with Cis led to decrease in serum urea and creatinine levels compared to EAC-bearing mice. This finding is consistent with data obtained by Salama et al. (2022) and El-Naggar et al. (2023). EAC-bearing mice treated with EDEP led to decrease in serum urea and creatinine levels which indicates that EDEP has protective effect on kidney against EAC cells. Treatment of EAC-bearing mice with combination of Cis/EDEP showed significant decrease in kidney biomarkers levels could be due to the synergistic effect between Cis and EDEP .

In this study, EAC-bearing mice showed a significant decrease in SOD and CAT activities while there was a significant increase in MDA level. A previous study by Ali et al. (2015) reported that EAC caused an increase in MDA levels with a decrease in SOD and CAT activities. This is due to the toxic effect of EAC on liver, the hepatocytes are under oxidative stress with active oxygen production by tumor cells causing antioxidant disturbance (Noda and Wakausugi, 2001). EAC-bearing mice treated with Cis showed increase in SOD and CAT activities with a decrease in MDA level when compared to EAC-bearing mice. Treatment with EDEP alone or with Cis showed a significant increase in SOD and CAT activities associated with decrease in MDA level. So far there are no previous studies reporting the antioxidant effect of EDEP. A Previous study by Ibrahim et al. (2022) who reported that *E. desertorum* mucin could act as antioxidant and anti-inflammatory agent for hepatic disorders against $CCl_4$ and Cis . The histopathological alterations noticed in the liver of EAC-bearing mice were represented by marked disorganization of hepatic structure, severe congested central veins, mostly hepatocytes were degenerated with vacuolated cytoplasm, dilated and widening blood sinusoids with distinct Kupffer cells and severe mononuclear infiltration was noticed. The current research was in agreement with the results of Attia et al. (2022) who reported that EAC cells can migrate from the peritoneal cavity and reach the liver causing liver injury. Liver section of mice that had treated with EAC/Cis showing improvement of the hepatic architecture represented by regular central vein, mostly hepatocytes are normal, few numbers are degenerated with vacuolated cytoplasm, others with abnormal nuclei like megakaryocytic ones, deteriorated and narrow blood sinusoids with normal Kupffer cells. Similar results were reported by El-Naggar et al. (2015). While liver section of EAC-bearing mice treated with EDEP and Cis exhibits improvement of the hepatic architecture, normal radiating hepatocytes with normal nuclei, enlarged central vein, few number of hepatocytes with vacuolated cytoplasm, regular blood sinusoids with normal Kupffer cells were noticed. Ibrahim et al. (2022) stated that the histopathological alterations that were caused by $CCl_4$ were improved after the treatment with *E. desertorum* snail mucin. This extract enhanced antioxidant activity and ameliorated the $CCl_4$-induced liver damage and it could be used as a hepatoprotective agent. Also, our results revealed that kidney sections of EAC-bearing mice induced histological

alterations represented by disorganization of the glomeruli, mostly renal tubules are disorganized and damaged, lost their characteristic appearance, as well as inter-tubular hemorrhage and aggregation of inflammatory cells were noticed. While kidney sections of mice treated with EDEP and Cis showed improvement in the renal architecture represented by normal glomeruli with regular bowman's space, mostly renal tubules were normal, but few number were damaged. Based on the scientific search, till now there are no previous studies reporting the the histopathological changes caused by EDEP on kidney sections.

In conclusion, treatment of EAC-bearing mice with EDEP did not show anti-tumor activity, however, the treatment with a combination of Cis/ EDEP improved the hepato-renal toxicities that induced by EAC inoculation.

## Conflicts of interest

There are no conflicts of interest to declare.

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