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Antitumor efficacy of the Egyptian scorpion *Leiurus quinquestriatus* whole body extract in EAC-bearing mice

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ABSTRACT

Cancer chemotherapy is an effective setting for treatments; however, it own severe side effects on vital organs. This study was conducted to evaluate the efficacy of *Leiurus quinquestratus* scorpion whole-body extract (LQWBE) as anticancer agent. Furthermore, the hepatic protective effect of LQWBE was evaluated. Forty female CD-1 mice were divided into four groups (n = 10). All groups were inoculated with $1x10^6$ EAC-cells/mouse. After 24 h of inoculation, group 1 (Gp1) left as EAC-bearing mice alone. Gp2 was injected with LQWBE (50 mg/Kg). Gp3 was injected with Cis (2 mg/Kg), while, Gp4 was injected with Cis/LQWBE as in Gp3 and Gp4. All injections were intraperitoneally (i.p), daily for 7 days. At day 14, the percentages of body weight changes (% b.wt), and tumor profile were determined. Hematological, biochemical, and histopathological investigations were assessed. The results showed that the effect of LQWBE had a moderate antitumor effect, however, provides a protective effect on hepatic tissues in EAC-bearing mice.

Keywords:

Antitumor, Scorpion, *Leiurus quinquestratus*, EAC-bearing mice

1. Introduction

Treatment approaches of cancer including chemotherapy, radiotherapy, surgery, and immunotherapy were applied. All the drawbacks presently associated with available chemotherapeutic agents are impetus for the search for newer, more efficacious, and better tolerated drugs (Marrotini and Pane, 2010). Chemotherapy treatments are effective against various types of cancers; however, a considerable proportion of patients often relapse due to drug resistance and/or toxicity to multiple organs (El-Sawalhi and Ahmed, 2014; Dasari et al., 2022). Therefore, finding newer, more efficacious, and better tolerated agents as anti-cancer drugs without harming cells or organs is ultimate .

Natural products play an important role in cancer therapeutics. These products have different bioactivities and variable bioavailability (Wang et al., 2014). Animal's bioactive compounds have been used as medicinal resources for the treatment of diseases (Mussarat et al., 2021; El-Feki et al., 2023). Natural extracts or venom of invertebrates have pharmaceutical activities such as anticancer, anti-diabetic, anti-microbial and anti-inflammatory agents (Cooper, 2012; Salama and Geasa, 2014; Salama et al., 2023) . Scorpions are terrestrial arachnids that are easily recognized by their characteristic elongated body and segmented tail ending in a bulbous sac and a stinger (Ozkan and Karear, 2007). In Egypt, there are 24 scorpion species, *L. quinquestriatus* is known by several names including the Egyptian Scorpion, five-keeled gold scorpion and Arabian death-stalker (Ben-Abraham et al., 2000). Fried scorpion is traditionally eaten in Shandong, China

(Matthew, 2008). The hazardous and life-threatening effects of scorpion envenoming, therapeutic properties of scorpion body parts and venom in ancient medicine have been utilized by humans for thousands of years (Petricevich, 2020). A previous study investigated the effect of the scorpion whole body extract as antidiabetic agents (Xie et al., 2011). This study was conducted to investigate the efficacy of the Egyptian scorpion whole body extract (LQWBE) as antitumor agent in vivo, in addition, to evaluate its role as hepato-protective agent in EAC-bearing mice.

2. Materials and Methods

Chemicals

Cisplatin was obtained from Sigma-Aldrich (St Quentin Fallavier, France). Vials were diluted with phosphate buffer saline (PBS) and the concentration was adjusted to 2 mg/kg body weight (b.wt) in 200 µL PBS. Kits for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were purchased from local company Bio-diagnostic (Egypt).

Collection of scorpions and whole-body extract preparation

One hundred of scorpion specimens were collected from Aswan, Egypt by professional hunters in July 2020. Scorpions were transferred in plastic containers to Invertebrate Division, Zoology Department, Faculty of Science, Tanta University, Egypt. Then, specimens were authenticated and identified by a specialist in animal taxonomy. To lower the toxic effect, the telsons containing venom glands were discarded from each scorpion. To prepare the *L. quinquestratus* whole-body extract (LQWBE), the scorpions were dried overnight at 60 °C and then grinded to obtain scorpion powder. The pooled powder was soaked in ethanol for 3 days then filtered. The filtrates were pooled and centrifuged (3000 rpm, 15 min) to remove impurities and debris. The supernatant of LQWBE was lyophilized and kept in −20 °C until use (Xie et al., 2011).

Mice and EAC cells inoculation

Female CD1 albino mice (22 ± 2 g) were obtained from Helwan University, after acclimatization for one week under the laboratory conditions. They were maintained under standard laboratory conditions (22 ± 1°C and 55 ± 5% relative humidity) and given the rodents food pellets (Egy Vet Care, El-Mahalla El-Kubra, Gharbia, Egypt) and water ad libitum. The animals were humanly treated, and the experimental design was approved by the animal care committee at the Zoology Department, Faculty of Science, Tanta University prior to perform the experiments (Protocol No.: IACUC-Sci- TU 0130).

The first inoculum of EAC cells was purchased from the Department of Tumor Biology, National Cancer Institute, Cairo University. EAC-cells were inoculated 1×10^6 cells / mouse intraperitoneal (i.p.). Cells were grown in the peritoneal cavity of mice. Mice were monitored daily for signs of tumor progression, including the amount of abdominal distension. The volume of ascites fluid was determined by needle (18–22 gauge) aspiration. Withdrawal of ascites fluid was performed under aseptic conditions.

Experimental design:

Forty female CD-1 mice were divided randomly into four groups (n = 10). All groups were inoculated with $1x10^6$ EAC-cells/mouse. After 24 hrs of inoculation, Gp1 was left as EAC-bearing mice alone. Gp2 were injected with LQWBE (50 mg/Kg), Gp3 were injected with Cis (2 mg/Kg), and Gp4 were injected with Cis/LQWBE. All injections were i.p., daily for 7 days. All groups were bled via the orbital plexus to collect blood samples for hematological and biochemical assessments. Liver tissues were fixed in buffered formalin for histopathological investigations.

Determination of body weight changes (%b.wt)

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

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

Determination of the hematological and biochemical parameters.

Red blood cells (R.B.Cs) count, hemoglobin (Hb) content, hematocrit (Hct%), platelets, and white blood cells (W.B.Cs) and their differential counts were determined by using the automatic blood counter (Mendary, China). Serum ALT, AST, SOD, CAT activities, and MDA levels were determined according to the manufacturer's instruction.

Liver histological examination

Liver tissues were collected from mice under appropriate anesthesia. Small pieces of liver tissues were immediately fixed in 10% neutral buffered formalin for 24 hrs. 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 5µm thickness were mounted and stained with hematoxylin and eosin method for histological examination (Bancroft and Gamble, 2002).

Statistical analysis

The data were expressed as mean ± standard deviation. 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 (Cairo, Egypt).

3. Results

Treatment with LQWBE decreased the % of body weight changes in EAC-bearing mice

Treatment with LQWBE decreased the % of body weight changes in EAC-bearing mice

The % b.wt change in the EAC-bearing mice was 48.7 % after 2 weeks. Treatment with LQWBE led to a decrease in the % b.wt changes to 44.7% when compared to EAC-bearing mice. The results showed that treatment with Cis led to a decrease in the final body weight with a percentage of change to -25.5 %. The % b.wt change in the EAC-bearing mice that injected with Cis/LQWBE was -36 % (Fig. 1) .

Effect of LQWBE on the tumor profile in EAC-bearing mice

Compared to the EAC-bearing mice, the treatment with LQWBE alone did not show a significant antitumor activity post 6 days of treatment. Treating EAC-bearing mice with Cis did show a significant decrease in tumor volume (0.55± 0.19 ml/mouse) with a reduction percentage (93.1%). Treatment with a combination with Cis/LQWBE reported similar finding to group of EAC-bearing mice treated with Cis alone (Table 1).

The number of the total EAC cells (TCC) was 560 ± 25 per mouse in EAC-bearing mice. Treatment with LQWBE, however, did not alter the number of EAC cells when compared to EAC-bearing mice alone. Treatment of EAC-bearing mice with Cis, however, led to significant decrease in TCC with a reduction percentage 93.7%. Treatment of EAC-bearing mice with a combination with Cis/LQWBE showed similar results to those treated with Cis alone. Treatment with Cis alone led to a significant decrease in the total EAC-life cells and increase in the total EAC-dead cells when compared to EAC-bearing mice (Table 1).

Treatment with LQWBE ameliorated the hematological alterations in EAC-bearing mice

The results showed that treatment of EAC-bearing mice with LQWBE did not significantly altered the R.B.Cs, Hb level, Hct %, and platelets count, when compared to EAC-bearing mice. The data reported that EAC-bearing mice that treated with Cis represented 9.5 $\times 10^6$/ul, 15.6 g/dl, 43.2, 840 $\times 10^3$/ul for R.B.Cs count, Hb level, Hct%, and platelets count, respectively (Table 2). W.B.Cs count was decreased in EAC-bearing mice treated with Cis when compare to EAC-bearing mice alone. Treating EAC-bearing mice with a combination of Cis/LQWBE, however, restore the W.B.Cs count values close to the normal levels (Table 3). Treatment of EAC-bearing mice with LQWBE did not show changes in the differential leucocyte's percentages, while treatment of EAC-bearing mice with Cis led to increase in the percentage of lymphocytes along with decreased neutrophiles, and monocytes. Treatment of EAC-bearing mice with a combination of Cis/LQWBE, however, restore the percentages of the differential leucocytes close to the normal levels (Table 3).

Treatment with LQWBE ameliorated liver dysfunctions in EAC-bearing mice

The results showed that the levels of liver transaminase (ALT, AST) in EAC-bearing mice that treated with LQWBE were 69.5 U/L, and 287 U/L, respectively. The liver enzymes parameters ALT and AST in EAC-bearing mice that treated with Cis were 184.5 U/L, and 332 U/L, respectively. Treatment of EAC-bearing mice with a combination of Cis/LQWBE, however, led to a decrease in the liver transaminases when compared to EAC-bearing mice (Fig. 2).

Effect of the treatment with Cis and/or LQWBE on the liver structures of EAC-bearing mice

A section in liver of EAC-bearing mice showed degeneration of hepatocytes with vacuolization, megakaryocytes, necrosis, hypertrophied Kupffer cells, and binucleated hepatocytes (Fig. 3A). The liver section of EAC-bearing mice treated with Cis showing improvement in architecture with dilation in blood sinusoids, binucleated hepatocytes, basophilic in filtration, and hypertrophied Kupffer cells (Fig. 3B). Fig. 3C showed a section in liver of EAC-bearing mice treated with LQWBE, showing less improvement, necrosis. The liver section of EAC bearing mice treated with Cis and LQWBE, showing more improvement of hepatocytes architecture like control group (Fig. 3D).

The immunohistochemistry (IHC) of caspase-3 (casp-3) protein in liver section of EAC-bearing mice was shown in figure 4. The intensity of casp-3 elevated when the apoptotic cell numbers increased. This intensity ranged from dark brown to yellowish brown color in the cytoplasm of liver. Over expression of Casp-3 in liver cytoplasm of EAC -bearing mice was shown and represented by the dark brown color that was spread out along the liver section (Fig. 4A). EAC-bearing mice that treated with Cis, showed moderate expression of Casp-3 in the cytoplasm of the apoptotic hepatocytes in the liver section (Fig. 4B). Whilst mild reduction in casp-3 expression in hepatocytes cytoplasm of EAC- bearing mice that treated with LQWBE (Fig. 4C). In contrast Casp-3 protein expression was noticeably decreased (yellowish brown color) in the hepatocyte's cytoplasm of EAC -bearing mice that treated with Cis and LQWBE (Fig. 4D).

Table 1. The tumor volume, tumor cells count, live and dead cancer cells in different groups.

Groups	T.V. (mL)		T.C.C x(10^6)		T.L.C x(10^6)		T.D.C x (10^6)	
	M ± SD	r%	M ± SD	r%	M ± SD	r%	M ± SD	r%
EAC control	1.4^{a}±8.0	-	25^{a}±560	-	531 ± 28^{a}	-	3.1^{a}±29	-
EAC/LQWBE	0.51^{b}±5.2	35	22.5^{b} ±360	35.7	24^{b}±340	35.9	$7.5^{a,b}$ ±20	31
EAC/Cis	0.19^{c} ±0.55	93.1	6.9^{c} ±35	93.7	6.0^{c}±18.0	96.6	$3.2^{a,b}$ ±17	41.3
EAC/Cis/ LQWBE	0.17^{c} ±0.50	93.7	5.8^{c} ±34	93.9	5.0^{c}±22.0	95.8	4.3^{b} ±12	58.6

EAC: Ehrlich ascitic carcinoma, Cis: Cisplatin, LQWBE: *L. quinquestratus* whole body ethanolic extract. Groups that don't share a letter are significantly different. r%: the percentage of reduction, T.V.: Total tumor volume, T.C.C.: Total tumor cell count, T.L.C.: Total live cells, T.D.C.: Total dead cells.

Table 2. The hematological parameters in different groups of EAC-bearing mice.

Groups	R.B.Cs (×10^6/ul)	Hb (g/dl)	Hct %	Platelets (×10^3/ul)
EAC control	7.9 ± 0.95^{a}	11.8 ± 1.08^{a}	32.4 ± 1.7^{b}	935 ± 52.5^{b}
EAC/LQWBE	8.2 ± 1.2^{a}	12.5 ± 2.1^{a}	34.6 ± 2.5^{b}	1454 ± 71^{a}
EAC/Cis	9.5 ± 1.5^{a}	15.6 ± 2.5^{a}	43.2 ± 3.5^{a}	840 ± 60^{b}
EAC/Cis/ LQWBE	9.7 ± 1.4^{a}	14.2 ± 1.5^{a}	35.1 ± 3.6^{b}	630 ± 44^{c}

LQWBE: *L. quinquestratus* whole body ethanolic extract. Groups EAC: Ehrlich ascitic carcinoma, Cis: Cisplatin, that don't share a letter are significantly different

Table 3. Differential leucocytes in different groups of EAC-bearing mice.

Groups	W.B.Cs ($\times10^3$/ul)	Lymph. %	Neut. %	Mono. %
EAC control	11.6 ± 3.5^{a}	38.3 ± 2.5^{c}	59 ± 0.59^{a}	3.6 ± 1.5^{a}
EAC/LQWBE	12.4 ± 2.3^{a}	38.0 ± 1.4^{c}	60 ± 1.02^{a}	2.0 ± 1.37^{a}
EAC/Cis	10.6 ± 2.7^{a}	48.0 ± 1.9^{b}	23 ± 1.60^{c}	2.8 ± 2.50^{a}
EAC/Cis/ LQWBE	15.0 ± 3.0^{a}	58.0 ± 0.57^{a}	40 ± 0.17^{b}	1.6 ± 0.57^{a}

EAC: Ehrlich ascitic carcinoma, Cis: Cisplatin, LQWBE: *L. quinquestratus* whole body ethanolic extract. Groups that don't share a letter are significantly different.

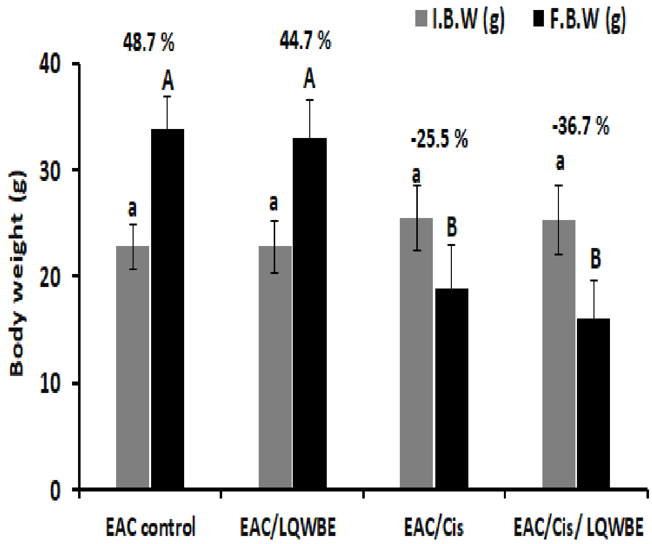

Fig. 1. Total body weight changes in EAC-bearing groups of mice.

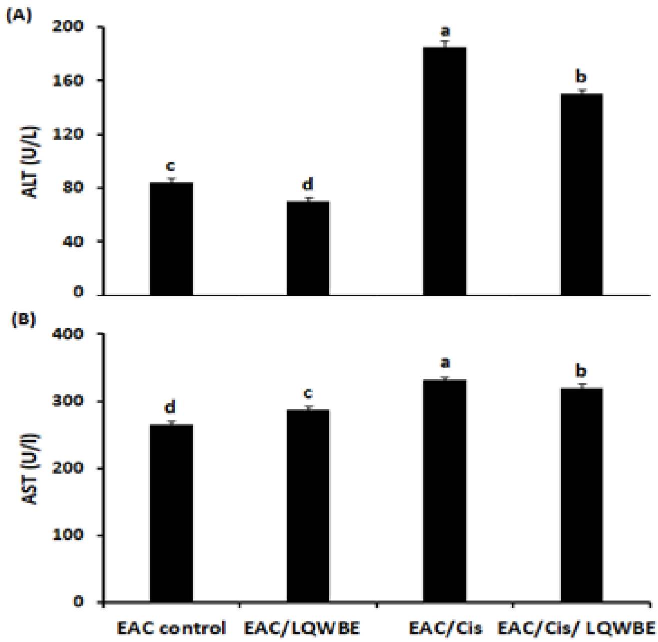

Fig. 2. Liver transaminases enzymes (A) Alanine transaminase (ALT) (B) Aspartate transaminase (AST) in the Ehrlich ascitic carcinoma (EAC) bearing mice groups.

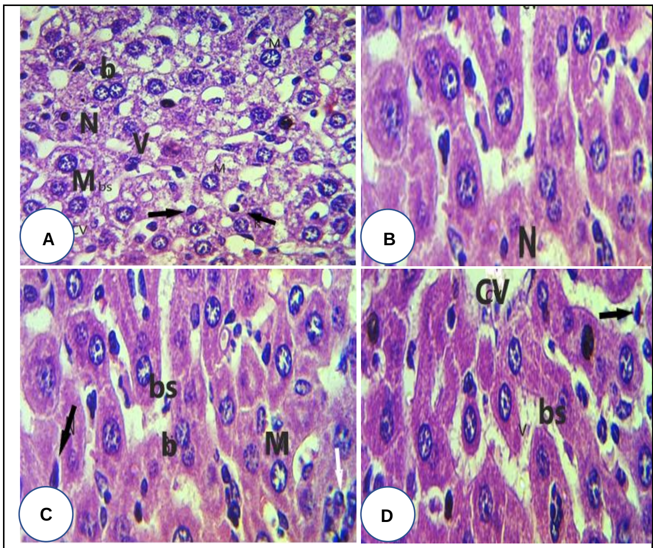

Fig. 3. Photomicrographs of the liver sections of the different EAC-bearing mice groups (A) EAC-bearing mice group showing degeneration of hepatocytes with vacuolization (v), megakaryocytes (M), necrosis (N), hypertrophied Kupffer cells (arrow), and binucleated hepatocytes (b), (B) EAC-bearing mice group treated with LQWBE, showing less improvement, necrosis (N), (C) EAC -bearing mice group treated with Cis showing improvement in architecture with dilation in blood sinusoids (bs), binucleated hepatocytes(b), basophilic in filtration (arrow), and hypertrophied Kupffer cells (black arrow), (D) EAC-bearing mice group treated with Cis and LQWBE, showing more improvement of hepatocytes architecture more or less similar to control group. Hematoxylin and Eosin, X400.

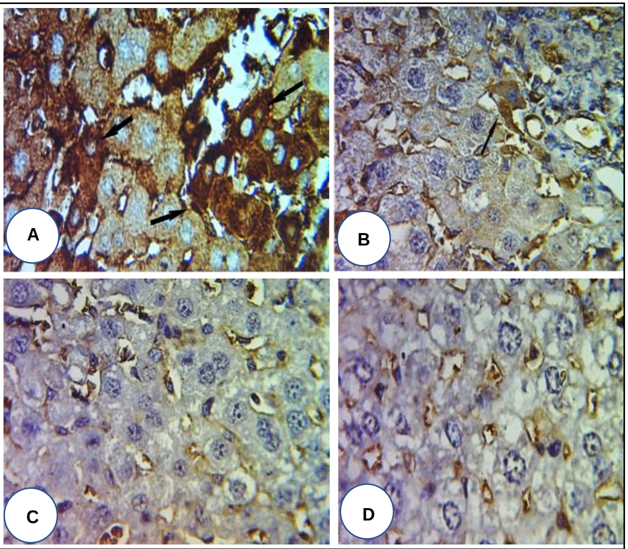

Fig. 4. Photomicrographs of the liver sections of the different EAC-treated mice expressing intense immunostin for Caspase-3. (A) EAC-treated mice showing the increment of apoptotic hepatocytes (arrow), (B) EAC/Cis expressing an obvious decline in the apoptotic cells. Few apoptotic hepatocytes express periphery to portal area (PA), (arrows) other beside the central vein (CV), (C) EAC bearing mice treated with LQWBE showing significant reduction in apoptotic cells caspase-3, (D) EAC-bearing mice treated with Cis/LQWBE, an obvious improvement decrement in the apoptotic cells and no immunostain to caspase-3 in most hepatic tissues periphery to central vein (cv), portal area (PA).

4. Discussion

Scorpions have plenty of uses especially in the biomedical fields (Salama and El-Naggar, 2021). Scorpion venom is currently in use in preclinical studies as antitumor, antileptic, antirheumatic agents. Recently, the whole body of scorpions was investigated as antidiabetic agent (Xie et al., 2011; Abdel-Rahmana et al., 2019). Chemotherapeutic drugs are used for the treatment of different malignancies, but their therapeutic use is limited due to their adverse side effects (Benzer et al., 2018). Animal's bioactive compounds have been used as medicinal resources for the treatment of diseases (Mussarat et al., 2021). Therefore, this study evaluated the effect of LQWBE as antitumor agent in EAC-bearing mice. Also, the hepatoprotective effect of LQWBE on EAC-bearing mice injected with Cis was assessed. The body weight of mice that injected with Cis decreased when compared to control group. This could explain the toxic effect of Cis on the vital organs in the body. Decreasing the relative organs weight post treatment with Cis confirm its toxicity. Treatment with a combination of Cis/LQWBE did not decrease the final body weight when compared to the group of mice that injected with Cis alone. This finding indicated that co-administration of LQWBE with Cis could protect the body's organs from Cis toxicity .

In EAC-bearing mice, anemia is mainly due to iron deficiency by hemolytic conditions accompanied by a decrease in RBCs' count (Sreelatha et al., 2011). The total R.B.Cs count, Hb level, Hct % were decreased in EAC-bearing mice. The total W.B.Cs count was increased in EAC-bearing mice, while, the treatment with Cis decreased the W.B.Cs count. Treatment with LQWBE in combination with Cis restored the number of W.B.Cs also to normal. This effect by LQWBE treatment could be due to the protection of the hematopoietic system (Nafie et al., 2020). ALT and AST are commonly used as markers of hepatocellular injuries. The progression of EAC-tumor in mice led to liver dysfunctions evidenced by increase of these enzymes. AST and ALT were elevated in serum of EAC–bearing mice (Bhattacharyya, 2007). Elevation of ALT and AST activities in EAC-bearing mice may be due to the cytotoxic effect of EAC tumors which led to damage of liver cells and canaculi. Cis increased ALT and AST in EAC-bearing mice (El-Naggar et al., 2016). The ALT and AST enzymes were decreased in the group of EAC-bearing mice treated with a combination of Cis/ LQWBE. Decreasing thehepatic toxicity upon treatment with this combination indicates that the LQWBE has a protective effect against liver dysfunction and cellular injury of liver. These results were further supported by liver histopathology. Co-treatment of EAC-bearing mice with Cis/LQWBE reduced the levels of serum urea and creatinine compared to EAC-bearing mice. This finding agreed with the previous reports of Thulfiqar and Tousson (2020) and Nafie et al. (2020). The LQWBE showed anticancer activity and ameliorate toxicities induced by tumor inoculation. The histological findings in the current study revealed that the injection of Cis in mice groups showed noticeable disorganized liver architecture with vacuolization and degeneration in hepatocytes

and the appearance of megakaryocytes, necrosis, and the activation of the hypertrophied Kupfer cells. Interestingly, increase in apoptotic cells in the cytoplasm of hepatocytes, hence over expression of Casp-3. Cisplatin forms inter- and intra-strand crosslinked DNA adducts and its cytotoxicity is mediated by propagation of DNA damage recognition signals to downstream pathways involving ATR, p53, p73, and mitogen-activated protein kinases (MAPK pathway), ultimately resulting in apoptosis (Tanida et al., 2012). Also, it has impacts on liver, as a major organ of detoxification. It is one of the most damaging alkylating agents that can cause oxidative stress due to the over-production of reactive oxygen species (ROS) (Palipoch and Punsawad, 2013) .

The liver architecture of the mice group that injected with LQWBE showed normal structure with weak expression of casp-3 in their cytoplasm. While, in the group of mice that injected with combination of Cis/LQWBE showed marked improvement in hepatic cellularity compared to that injected with Cis only. The hepatocytes retained their arrangement with slight kuppfer cells activation, binucleated and necrotic cells. This improvement also extends to the mild expression of Casp-3 in immunohistochemistry. The hepatoprotective effect obtained post treatment with LQWBE could be due to the effect of octadecanoic and oleic acids as anti-inflammatory and antioxidant activities (Zhang et al., 2015)

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