

RESEARCH ARTICLE

**THE CYTOTOXIC EFFECT OF RHIZOSTOME JELLYFISH VENOM  
ON CANCER CELL LINES AND ITS ANTITUMOR ACTIVITY  
IN EHRLICH ASCITES-BEARING MICE**

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**ABSTRACT**

All of the drawbacks associated with the chemotherapy of cancer are the impetus for the search for newer and more efficacious drugs. Therefore, the current study aimed to evaluate the cytotoxic and antitumor efficacy of scyphozoan *Rhizostoma octopus* (rhizostome jellyfish) venom extract (RVE) against different tumor cell lines (*in vitro*) and Ehrlich ascites carcinoma (EAC)-bearing mice (*in vivo*), respectively. Four groups of female CD-1 mice (n=10) were allotted as follows: group-1 (Gp1) was served as control. Gp2-Gp4 were injected intraperitoneally (i.p) with  $1 \times 10^6$  EAC cells/mouse. After 24 hours, Gp3 and Gp4 were injected (i.p) with cisplatin (2 mg/kg) or with 1/10 LD<sub>50</sub> of RVE (180 mg/kg body weight, b.wt) daily for 6 consecutive days, respectively. On day 14, the b.wt change, tumor indices, hematological and biochemical parameters, as well as histological alterations of liver and kidneys tissues, were investigated. The results showed that RVE had high protein content with six bands ranging between 15-70 kDa, as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The IC<sub>50</sub> of RVE against human hepatocellular (HepG-2) and breast cancer (MCF-7) cell lines were 808.4 and 896.4 µg/mL, respectively. In EAC-bearing mice, RVE treatment modulated the b.wt change, and decreased the tumor volume and the tumor cells count. In addition, the liver and kidney functions were improved in EAC-bearing mice treated with RVE, as evidenced by ameliorating their histological structure.

**Article History:**

Received: 5 April 2022

Accepted: 15 April 2022

Published Online:

5 May 2022

**Keywords:**

Antitumor activity

EAC-bearing mice

HepG-2

MCF-7

*Rhizostoma octopus*

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**INTRODUCTION**

Cancer is characterized by the uncontrolled multiplication of cells and is known to be the second cause of death after cardiovascular disease all over the world<sup>[1,2]</sup>. Different settings have been employed for cancer treatment including surgery, chemotherapy, radiotherapy, gene therapy, and immunotherapy<sup>[3-5]</sup>. Chemotherapy is used to treat patients that have metastasized

cancer to other parts of the body<sup>[6]</sup>. In the past decade, chemotherapeutic agents such as cisplatin (Cis) and paclitaxel were used for pre-conditioning the host before to the treatment with the cellular elements of the immune system for lung cancer<sup>[5]</sup>. The chemotherapeutic drug "Cis" is used to treat different malignancies<sup>[3,4]</sup>. Despite its potential effect, Cis has significant adverse effects on vital organs, including the kidney

and liver<sup>[7]</sup>. The repeated treatment with Cis causes resistance of tumor cells<sup>[8]</sup>. All of the drawbacks that were associated with Cis treatments are the impetus for the search for newer, more efficacious, and better tolerated drugs. Natural compounds have been used as potential therapeutic agents to treat different diseases including cancer<sup>[9-11]</sup>. New approaches for using natural products extracted from medicinal plants, microorganisms, and animal sources as anticancer drugs without harming the vital organs are the ultimate need<sup>[10-12]</sup>. Despite the abundance of marine invertebrates, their pharmacological uses are limited<sup>[13]</sup>. Several natural marine compounds were tested against the cardiovascular, endocrine, immune, and nervous systems disorders, as well as anti-inflammatory and antitumor activities were evaluated<sup>[14]</sup>.

Organisms in phylum Cnidarians are important due to their ability to produce natural substances such as venom from special structure called nematocyst for defense purposes. Venom's complex secretion consists of enzymes, neurotoxins, and pore-forming toxins<sup>[15]</sup>. A previous study showed that the bioactive compounds extracted from cnidarians venom have several therapeutic effects, for instance, prostaglandins and palytoxin extracted from the Anthozoan *Plaxaura homomalla*, and *Palythoa toxica*, respectively, were used as a local anesthetic and vasoconstrictive agents<sup>[13]</sup>. Furthermore, prostanoid compounds isolated from the *Clavularia viridis* have revealed an antitumor effect on leukemic cells (HL-60) by inhibiting their growth<sup>[16]</sup>. Equinatoxin extracted from *Actinia equina* showed antitumor activity on cancer cells *in vitro*<sup>[13]</sup>. A previous study has also reported that the *Cassiopea xamachana* venom has a potent antitumor effect<sup>[17]</sup>.

The jellyfish population has increased in seas and oceans as a result of global warming, climate, and salinity changes in marine water, for instance, the lessepsian migration of marine organisms across the Suez Canal in Egypt<sup>[18]</sup>. *Rhizostoma*

*octopus* (rhizostome jellyfish) is a scyphozoan jellyfish that belongs to the class Scyphozoa, order Rhizostomeae. It is the largest scyphomedusa that inhabits the Egyptian Mediterranean Sea<sup>[19]</sup>. Previous studies have evaluated the biomedical importance of jellyfish as antifatigue, antioxidant, antimicrobial, insecticidal, immune-stimulative, anticoagulant, anti-hemolytic, anticardiovascular disorder, anti-hypertensive, antianalgesic, and hypocholesterolemic agents<sup>[20-22]</sup>. Few studies reported the anti-tumor effect of jellyfish and soft corals extracts against the murine tumor. Therefore, this study aimed to evaluate the cytotoxic effect of the scyphozoan *R. octopus* venom extract (RVE) on human hepatocellular (HepG-2) and breast cancer (MCF-7) cell lines *in vitro*, in addition to determine its antitumor activity in Ehrlich ascites carcinoma (EAC)-bearing mice.

## MATERIAL AND METHODS

### Chemicals

The chemotherapeutic agents "Cis and "doxorubicin (Dox)", dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and trypan blue dye were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution was purchased from Lonza (Bornem, Belgium). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine kits, as well as phosphate-buffered saline (PBS) were purchased from Bio-diagnostic Company (Giza, Egypt). For SDS-PAGE analysis, an unstained protein standard marker (10-200 kDa) was purchased from New England Biolabs (Ipswich, MA, USA).

### Collection and preparation of *R. octopus* venom extract (RVE)

Twenty specimens of Scyphomedusa (Cnidaria, Scyphozoa, Rhizostomeae) were

collected from the Mediterranean Sea, Miami station, Alexandria (31.27 latitudes and 29.98 longitudes, 31°C). The specimens were identified, described, and authenticated by a taxonomist at the Zoology Department, Faculty of Science, Tanta University. A modified method of Jouiaei *et al.*<sup>[15]</sup> was used to prepare RVE. Briefly, *R. octopus* oral arms were immersed in 1.0 L absolute ethanol for 30 seconds at room temperature to promote the chemical discharge of nematocysts. Then, the extract was left for 24 hours at 4°C to be precipitated. The precipitate was centrifuged for 30 minutes at 14000 ×g and 4°C. The lyophilized RVE was kept at -80°C until use.

#### **Biochemical assays and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of RVE**

The total protein, lipids, and carbohydrates contents of RVE were calculated according to Stoscheck<sup>[23]</sup>, Folch *et al.*<sup>[24]</sup>, and Plummer<sup>[25]</sup>, respectively. SDS-PAGE (12% polyacrylamide gel) analysis was performed according to Laemmli<sup>[26]</sup>. The molecular weights were calculated using KODAK Molecular Imaging Software 5.0 (MIS, Carestream Health, Inc., New Haven, CT, USA).

#### **Human cancer cell lines**

Cancer cell lines “HepG-2 and MCF-7” were obtained from VACSERA (Giza, Egypt). Cancer cell lines were seeded in DMEM supplemented with 10% fetal bovine serum (BioWest, Nuaille, France), 100 U/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL glutamine at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cells were sub-cultured every two days.

#### **In vitro cytotoxic assay by MTT**

The concentration of RVE that inhibit 50% (IC<sub>50</sub>) of HepG-2 and MCF-7 cancer cell proliferation was determined by using the MTT assay. Briefly, different concentrations of RVE (3.9-2000 µg/mL) were applied in triplicate to treat these cells. The treated cells were incubated for 24 hours; then, 10 µL of a 12 mmol MTT stock solution

(5 mg/mL) in PBS was added to each well, followed by incubation for 4 h at 37°C. The MTT solution was removed, and the formed purple formazan crystals were dissolved with 100 µL of DMSO for 20 min. Dox was used as a positive reference drug in MTT Assay. The absorbance at 570 nm was determined by an ELISA reader (StatFax-2100, Awareness Technology, Inc., Palm City, FL, USA). IC<sub>50</sub> was calculated with the sigmoidal curve<sup>[27]</sup>.

#### **Mice**

Female CD-1 mice (20±4 g) were obtained from National Research Center (NRC, Cairo, Egypt). Mice were kept for a week for adaptation. The temperature and relative humidity were about 22±1°C and 55±5%, respectively. Light-dark (day/night) cycle was achieved and the animals were handled according to the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University (Protocol number: IACUC-SCI-TU 0186).

#### **Determination of the median lethal dose (LD<sub>50</sub>) of RVE in mice**

Twenty-four female CD-1 mice were divided into six groups (n=4). These groups were injected intraperitoneal (i.p) with a single dose of 1.0-6.0 g/kg of RVE and were monitored for 24 hours to assess the LD<sub>50</sub>. RVE LD<sub>50</sub> value was calculated using the probit analysis<sup>[28]</sup>.

#### **EAC cells inoculation and experimental design**

EAC cells were inoculated (i.p) with 1×10<sup>6</sup> cells/mouse. Then, mice were monitored and the ascites fluid was drawn under aseptic conditions and its volume was determined. Forty female CD-1 mice were allotted randomly into four groups (n=10) as follows: All groups except group “1” (Gp1) had inoculated (i.p) with 1×10<sup>6</sup> EAC cells/mouse in 200 µL sterile saline. After 24 hours, Gp2 was left as untreated EAC-bearing mice. Gp3 and Gp4 received i.p injection of Cis (2 mg/kg) or RVE (180 mg/kg), daily for 6 consecutive days, respectively. At the

beginning and the end of the experiment, the initial body weight (b.wt) and the final b.wt were determined, respectively. By day 14, mice were bled under appropriate anesthesia (isoflurane 100%, Pharco Pharmaceuticals, Alexandria, Egypt) to collect blood samples for hematological and biochemical assessments. Liver and kidney were immediately separated and fixed in 10% neutral buffered formalin for histological investigations.

#### **Determination of the complete blood picture and the biochemical parameters**

The total red blood cells (RBCs) count, hemoglobin (Hb) content, hematocrit value (Hct %), the total platelets count, total white blood cells (WBCs) count and their differential counts were determined by using the Mindary automatic blood counter (Guangzhou, Guangdong, China). Serum aminotransferases (ALT and AST) activities and kidney functions (urea and creatinine) were determined by colorimetric methods according to the Bio-diagnostic instruction.

#### **Liver and kidney histopathological examinations**

The fixed tissue samples were dehydrated in ascending grades of ethyl alcohol and embedded in paraffin wax. Sections of 5  $\mu\text{m}$  thickness were mounted and stained with hematoxylin and eosin for histological examination<sup>[29]</sup>.

#### **Statistical analysis**

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

## **RESULTS**

#### **Morphometric characterization of the scyphozoan specimen**

The collected specimens were identified as the scyphozoan *R. octopus* (Linnaeus, 1788)

that belongs to order Rhizostomeae. The results showed that the *R. octopus* body was hemispherical without marginal tentacles and consisted of two main parts: the umbrella or bell and the oral cone. The umbrella was subdivided into a central thick ex-umbrella and peripheral thin sub-umbrella, which end with blue-purple pigmented marginal lappets. The mouth was surrounded by eight oral arms hanged centrally from a sub-umbrella. The scapulets were the base of oral arms, which had funnel projections called suctorial mouths. The oral arms were ended with three winged appendages or club structures (Figure 1). The results showed that the bell diameter and weight of *R. octopus* (Linnaeus, 1788) were  $38 \pm 2$  cm, and  $2.00 \pm 0.17$  kg, respectively.

#### **Biochemical compositions and protein profile of RVE**

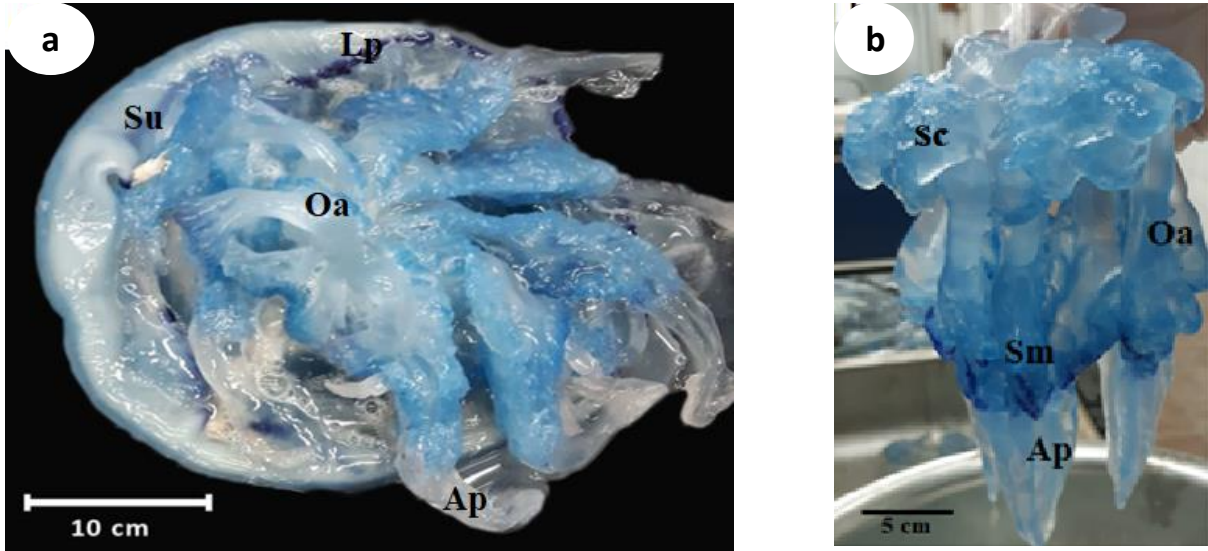
The results showed that total protein, total lipid, and total carbohydrate contents of RVE were  $44.6 \pm 1.5$ ,  $12.2 \pm 0.4$  and  $0.03 \pm 0.01$  mg/g of dry weight, respectively. SDS-PAGE protein profile of RVE showed the presence of six protein bands with molecular weights ranging between 15-70 kDa as low molecular weights protein bands (Figure 2).

#### **The LD<sub>50</sub> value of RVE**

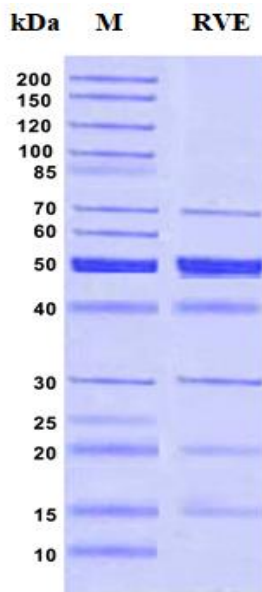
After 24 hours of injection with different doses of RVE, the mortality percentages of the female CD-1 mice were calculated. Then, LD<sub>50</sub> was calculated using probit analysis. The results showed that the LD<sub>50</sub> of RVE was 1.8 g/kg b.wt (Figure 3).

#### **Cytotoxic effect of RVE against HepG-2 and MCF-7 cells *in vitro***

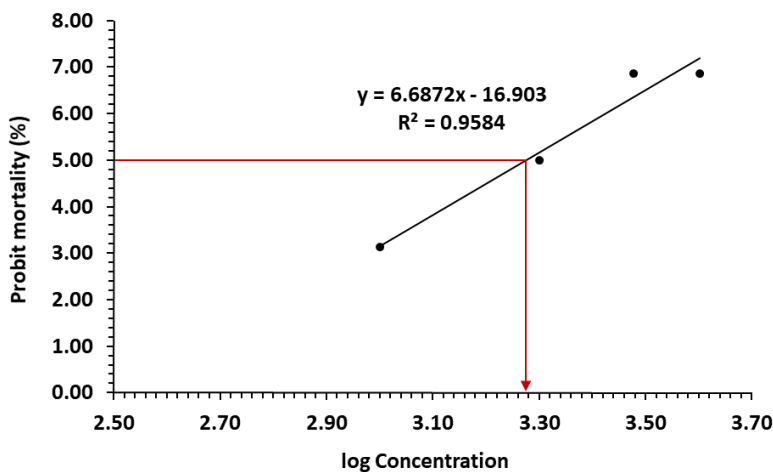
The IC<sub>50</sub> of RVE was determined *in vitro* against HepG-2 and MCF-7 cell lines by MTT assay. The data showed that the RVE IC<sub>50</sub> against HepG-2 and MCF-7 cells after 24 hours were  $808.4 \pm 31$  and  $896.3 \pm 38.9$   $\mu\text{g/mL}$ , respectively. The IC<sub>50</sub> of Dox against HepG-2 and MCF-7 cells were  $0.37 \pm 0.05$  and  $0.36 \pm 0.04$   $\mu\text{g/mL}$ , respectively (Figure 4).



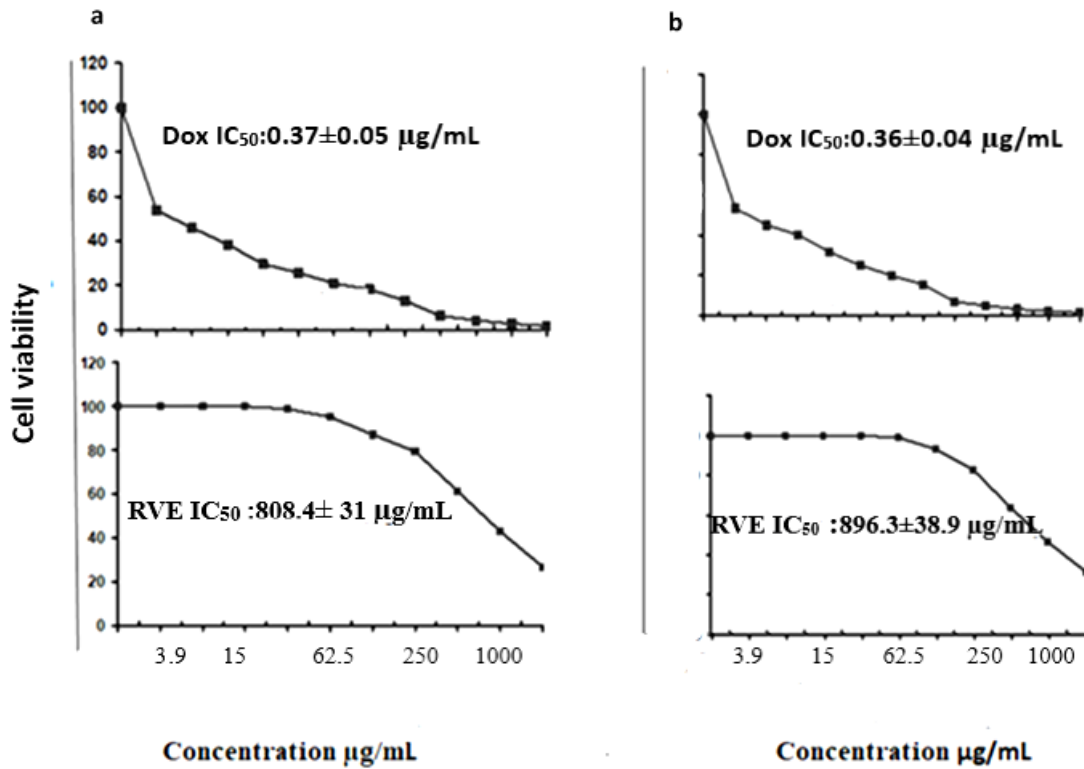
**Figure 1:** Oral view of *Rhizostoma octopus* (Linnaeus, 1788) showing (a) subumbrella (Su) end with blue pigmented marginal lappet (Lp) and oral arms (Oa) that end with 3 winged appendages (Ap) (scale bar = 10 cm), (b) isolated oral arms (Oa) of *R. octopus* with scapulets (Sc), suctorial mouths (Sm) and three winged appendages (Ap) (scale bar = 5 cm).



**Figure 2:** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of *R. octopus* venom extract (RVE). M: protein standard marker (10-200 kDa, Biolaps, England).



**Figure 3:** The median lethal dose (LD<sub>50</sub>) of *R. octopus* venom extract on mice after 24 hours using probit analysis.

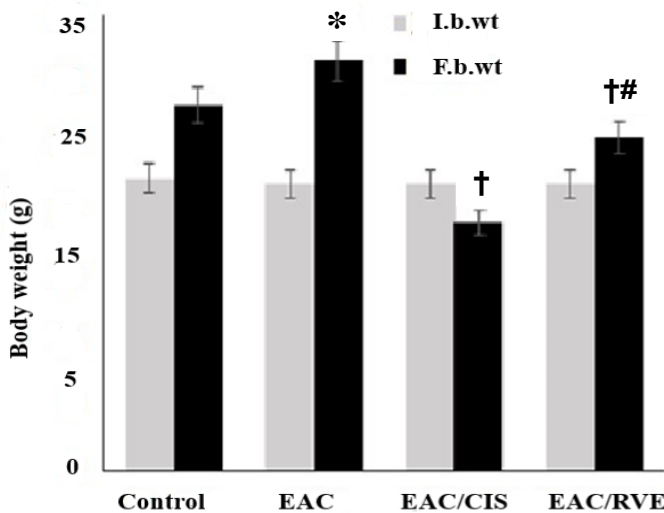


**Figure 4:** The median inhibition concentration (IC<sub>50</sub>) of *R. octopus* venom extract (RVE) on HepG-2 (a) and MCF-7 (b) cells compared with reference drug doxorubicin (Dox) after 24 hours.

**Treatment with RVE decreased the percentage of b.wt change in EAC-bearing mice**

The data represented in Figure (5) showed that there was no significant change in initial b.wt of all groups under the study. On day 14, the final b.wt in the group of mice inoculated with EAC alone showed a significant increase ( $P < 0.05$ )

when compared with their control. In contrast, the final b.wt was decreased significantly ( $P < 0.05$ ) in the group of EAC-bearing mice treated with Cis or RVE, as compared with the EAC-bearing mice. However, treatment of EAC-bearing mice with RVE increased significantly ( $P < 0.05$ ) the final b.wt when compared with EAC-bearing mice treated with Cis.



**Figure 5:** Initial body weight (I.b.wt) and final body weight (F.b.wt) of the different group under the study. EAC: Erlich ascites carcinoma-bearing mice, EAC/Cis: EAC-bearing mice treated with cisplatin (Cis), EAC/RVE: EAC-bearing mice treated with *Rhizostoma octopus* venom extract (RVE), \* $P < 0.05$ : significant compared with F.b.wt of the control group. † $P < 0.05$ : significant compared with F.b.wt of EAC-bearing mice. # $P < 0.05$ : significant compared with F.b.wt of EAC/Cis group.

### Effect of RVE treatment on the tumor profile in EAC-bearing mice

Treatment of EAC-bearing mice with Cis showed significant decreases ( $P<0.05$ ) in the tumor volume ( $0.56\pm 0.19$  mL/mouse) and the total count of tumor cells ( $35\pm 4.9\times 10^6$ /mouse) when compared with the EAC-bearing mice. Furthermore, treatment of EAC-bearing mice with RVE decreased significantly the tumor volume ( $3.26\pm 1.1$  mL/mouse) and the total

number of EAC-cells ( $190\pm 8.6\times 10^6$ /mouse) when compared with the EAC-bearing mice. The treatment with Cis or RVE led to a significant decrease ( $P<0.05$ ) in the live EAC cells count and the dead EAC cells count ( $P<0.05$ ) when compared with their counts in EAC-bearing mice. The data showed also that the effect of RVE in EAC-bearing mice was less than that of Cis (Table 1).

**Table (1):** Tumor volume, the total count of tumor cells, and the counts of live and dead tumor cells among the different groups.

Groups	Tumor volume (mL/mouse)	Total count of tumor cells	Live tumor	Dead tumor
			cell count	cell count
			( $\times 10^6$ /mouse)	
EAC	$8.20 \pm 2.30^a$	$560.0 \pm 25.0^a$	$531.0 \pm 28.0^a$	$29.0 \pm 3.1^a$
EAC/Cis	$0.56 \pm 0.19^c$	$35.0 \pm 4.9^c$	$17.0 \pm 2.0^c$	$18.0 \pm 1.2^{b,c}$
EAC/RVE	$3.26 \pm 1.10^b$	$190.0 \pm 8.6^b$	$170.0 \pm 24.0^b$	$20.0 \pm 2.2^b$

The values represented mean  $\pm$  standard deviation. EAC: EAC-bearing mice, EAC/Cis: EAC-bearing mice treated with cisplatin (Cis, 2 mg/kg), EAC/RVE: EAC-bearing mice treated with *Rhizostoma octopus* venom extract (RVE, 180 mg/kg). Means that do not share a letter in the same column are significantly different.

### Effect of RVE treatment on the total blood picture of EAC-bearing mice

The results showed that the total RBCs count, Hb content, Hct value, and the total platelets count were decreased significantly ( $P<0.05$ ) in the non-treated EAC-bearing mice when compared with their values in the control group. Treatment of EAC-bearing mice with Cis did not show significant differences in the total RBCs count, Hb content, and Hct value ( $P\geq 0.05$ ), while the total platelets count show a significant increase ( $P<0.05$ ) when compared with their values in EAC-bearing mice. EAC-bearing mice treated with RVE showed significant increases ( $P<0.05$ ) in the total RBCs count and Hb content, when compared with EAC-bearing mice. However, the Hct value and the total platelets count did not alter significantly ( $P\geq 0.05$ ) post RVE treatment when compared with EAC-bearing mice. Treatment of EAC-bearing mice with RVE

increased the total RBCs count, Hb content, and HCT value insignificantly, and decreased the total platelets count significantly when compared with EAC-bearing mice that were treated with Cis (Table 2).

The data reported a significant increase ( $P<0.05$ ) in the total WBCs count in EAC-bearing mice ( $11.6\pm 3.0\times 10^3/\mu\text{L}$ ) as compared with the count in the control group ( $7.45\pm 2.1\times 10^3/\mu\text{L}$ ). Treatment of EAC-bearing mice with Cis or RVE decreased significantly ( $P<0.05$ ) the total WBCs count ( $P<0.05$ ) when compared with its value in EAC-bearing mice (Table 3). In EAC-bearing mice, the absolute counts of the lymphocytes, neutrophils, and monocytes were increased significantly ( $P<0.05$ ) compared with these counts in the control group. In EAC-bearing mice treated with Cis or RVE, the absolute counts of these subsets were decreased significantly

( $P < 0.05$ ) when compared with their counts with EAC-bearing mice. However, the absolute count of lymphocytes showed a significant increase in EAC-bearing mice treated with RVE compared with its count in EAC-bearing mice treated with Cis (Table 3).

**Table (2):** Total red blood cell count (RBCs), hemoglobin (Hb) content, hematocrit (Hct) value, and the total platelets count among different groups.

Groups	RBCs ( $\times 10^6/\mu\text{L}$ )	Hb (g/dL)	Hct (%)	Platelets ( $\times 10^3/\mu\text{L}$ )
Control	$8.4 \pm 1.0^a$	$13.8 \pm 1.1^a$	$34.4 \pm 1.7^a$	$935.0 \pm 52.5^a$
EAC	$6.0 \pm 0.9^b$	$9.3 \pm 1.1^b$	$29.4 \pm 1.7^b$	$740.0 \pm 52.5^b$
EAC/Cis	$6.6 \pm 1.4^b$	$10.5 \pm 2.5^{a,b}$	$30.2 \pm 3.5^{a,b}$	$845.4 \pm 44.0^a$
EAC/RVE	$7.2 \pm 1.3^{a,b}$	$12.0 \pm 2.1^a$	$32.6 \pm 2.5^{a,b}$	$790.0 \pm 71.0^b$

The values represented mean  $\pm$  standard deviation. EAC: EAC-bearing mice, EAC/Cis: EAC-bearing mice treated with cisplatin (Cis, 2 mg/kg), EAC/RVE: EAC-bearing mice treated with *Rhizostoma octopus* venom extract (RVE, 180 mg/kg). Means that do not share a letter in the same column are significantly different.

**Table (3):** Total and differential counts of white blood cells (WBCs) among different groups.

Groups	WBCs	Lymphocytes	Neutrophils	Monocytes
	( $\times 10^3/\mu\text{L}$ )			
Control	$7.5 \pm 2.1^b$	$5.49 \pm 0.44^b$	$1.87 \pm 0.03^{b,c}$	$0.16 \pm 0.04^b$
EAC	$11.6 \pm 3.0^a$	$7.1 \pm 0.5^a$	$4.4 \pm 0.11^a$	$0.3 \pm 0.06^a$
EAC/Cis	$5.4 \pm 2.3^{c,d}$	$3.4 \pm 0.27^d$	$2.15 \pm 0.08^b$	$0.15 \pm 0.01^b$
EAC/RVE	$6.8 \pm 2.1^{b,c}$	$4.85 \pm 0.16^{b,c}$	$1.84 \pm 0.01^{b,c}$	$0.14 \pm 0.02^b$

The values represented mean  $\pm$  standard deviation. EAC: EAC-bearing mice, EAC/Cis: EAC-bearing mice treated with cisplatin (Cis, 2 mg/kg), EAC/RVE: EAC-bearing mice treated with *Rhizostoma octopus* venom extract (RVE, 180 mg/kg). Means that do not share a letter in the same column are significantly different.

#### Effect of RVE on serum amino-transferases activities and kidney functions in EAC-bearing mice

In EAC-bearing mice, serum ALT and AST activities were  $97.4 \pm 5.32$  and  $209.3 \pm 2.5$  U/L respectively. Serum ALT and AST activities, as well as urea and creatinine levels, were increased significantly ( $P < 0.05$ ) in EAC-bearing mice compared with their values in the control group. Treatment of EAC-bearing mice with Cis or RVE decreased significantly the elevation in the previous

biomarkers when compared with their levels in non-treated EAC-bearing mice (Table 4).

#### Effect of RVE on the histopathological changes in liver and kidneys in EAC-bearing mice

The liver sections of control mice showed normal hepatic architecture. The hepatic lobules appeared with hepatic strands separated by blood sinusoids surrounded the central vein (Figure 6a). The liver sections of EAC-bearing mice showed hepatocytes



degeneration, loss of cell boundaries, megakaryocytes, binucleated hepatocytes, and aggregations of basophilic and Kupffer cells (Figure 6b). Liver sections of EAC-bearing mice that were treated with Cis, however, showed an improvement in the architecture of the hepatic lobules with normal hepatic strands. In addition, the basophilic infiltration was reported around the dilated central vein and hypertrophied

Kupffer cells in these sections (Figure 6c). Liver sections of EAC-bearing mice that were treated with RVE, showed less improvement in liver architecture than those found in EAC-bearing mice that were treated with Cis. Binucleated hepatocytes, hepatocellular necrosis, and loss of cell boundaries, as well as basophilic infiltration around the central vein have appeared in RVE treated mice (Figure 6d).

**Table (4):** Serum aminotransferases (ALT and AST) activities, and urea and creatinine levels among different groups.

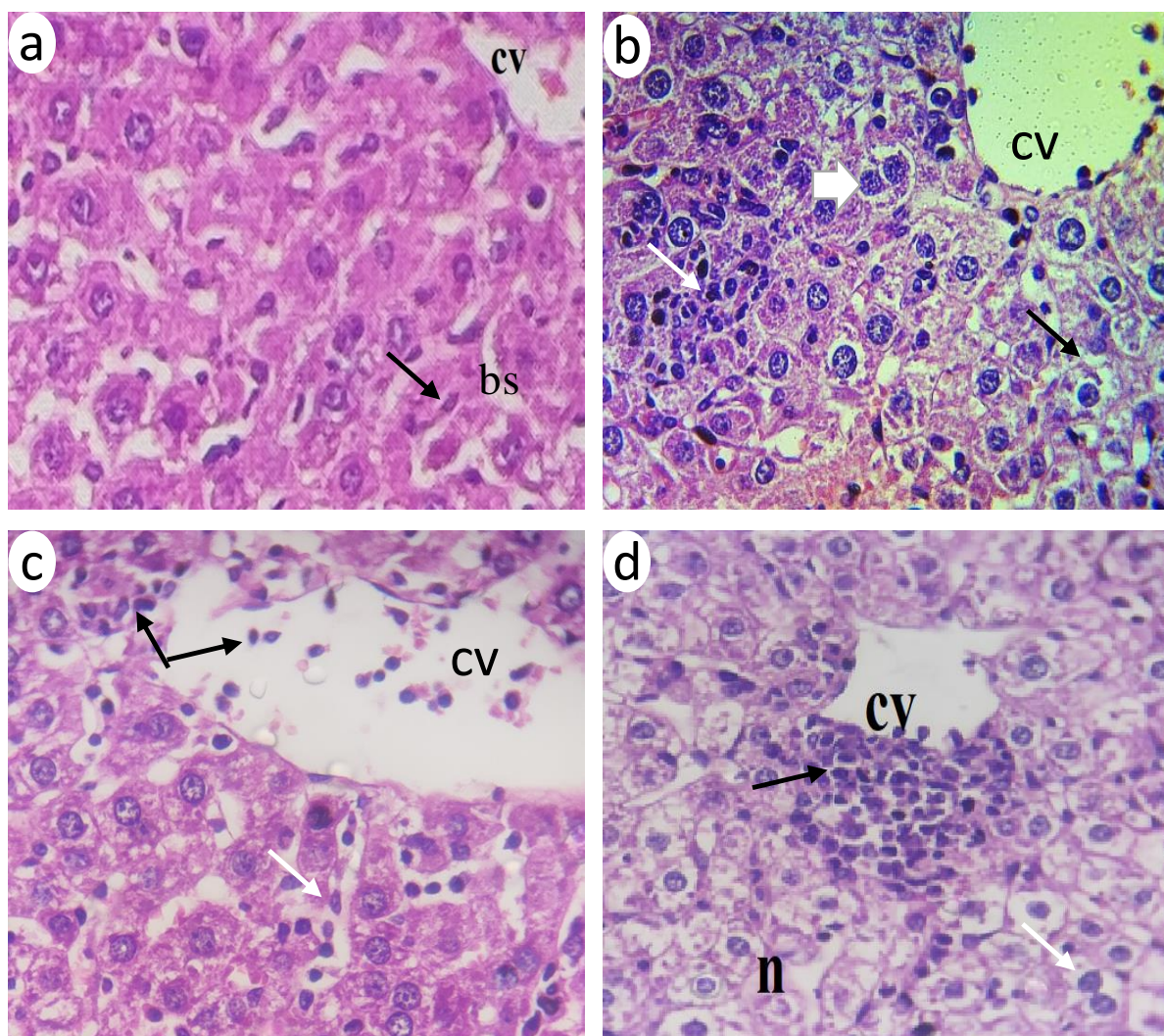
Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	34.8 ± 3.5 <sup>d</sup>	73.6 ± 3.5 <sup>d</sup>	20.2 ± 1.5 <sup>d</sup>	0.48 ± 0.01 <sup>d</sup>
EAC	97.4 ± 5.3 <sup>a</sup>	209.3 ± 2.5 <sup>a</sup>	56.4 ± 0.6 <sup>a</sup>	1.60 ± 0.05 <sup>a</sup>
EAC/Cis	60.3 ± 2.2 <sup>c</sup>	150.4 ± 1.9 <sup>c</sup>	43.8 ± 1.6 <sup>b,c</sup>	0.90 ± 0.02 <sup>c</sup>
EAC/RVE	84.7 ± 2.2 <sup>b</sup>	175.6 ± 1.4 <sup>b</sup>	49.5 ± 1.0 <sup>b</sup>	1.10 ± 0.07 <sup>b</sup>

The values represented mean ± standard deviation. EAC: EAC-bearing mice, EAC/Cis: EAC-bearing mice treated with cisplatin (Cis, 2 mg/kg), EAC/RVE: EAC-bearing mice treated with *Rhizostoma octopus* venom extract (RVE, 180 mg/kg), ALT: alanine aminotransferase, AST: aspartate aminotransferase. Means that do not share a letter in the same column are significantly different.

Kidney sections of control mice showed the normal structure of the cortical tissue that was composed from convoluted renal tubules (distal and proximal) and normal renal Bowman`s capsule surrounded the mass of blood capillaries called glomerulus (Figure 7a). Kidney sections of EAC-bearing mice illustrated deformation in the cortical tissues structure as well as degeneration, disappearance, or atrophy in glomeruli. In addition, there were vacuolization in renal tubules, and multifocal mononuclear cells infiltration between the renal tubules (Figure 7b). Treatment of EAC-bearing mice with Cis led to an improvement in the renal structure with a marked dilatation in the renal tubules and hypertrophy of glomerulus cells (Figure 7c). The treatment with RVE caused a dilatation in the Bowman`s space, vacuolization in the renal tubules, glomerulus hypertrophy, and interstitial hemorrhages (Figure 7d).

## DISCUSSION

The chemotherapeutic drug “Cis” is used for the treatment of different malignancies, however, the therapeutic effect is limited due to their adverse effects on the vital organs<sup>[30]</sup>. Several natural compounds that have been extracted from animal sources were evaluated for different pharmacological applications<sup>[31]</sup>. Despite the abundance and variations of marine invertebrates, the investigation of these organisms as sources for biomedical applications are limited<sup>[32]</sup>. The present study was conducted to characterize the chemical composition and the antitumor effect of RVE. The current study showed that the diameters and the weight of the collected *R. octopus* were 38±2 cm, and 2.00±0.17 kg, respectively. A previous study showed that the bell diameter of *R. octopus* ranged from 40 to 90 cm<sup>[33,34]</sup>. In the present study, the chemical compositions of RVE showed the



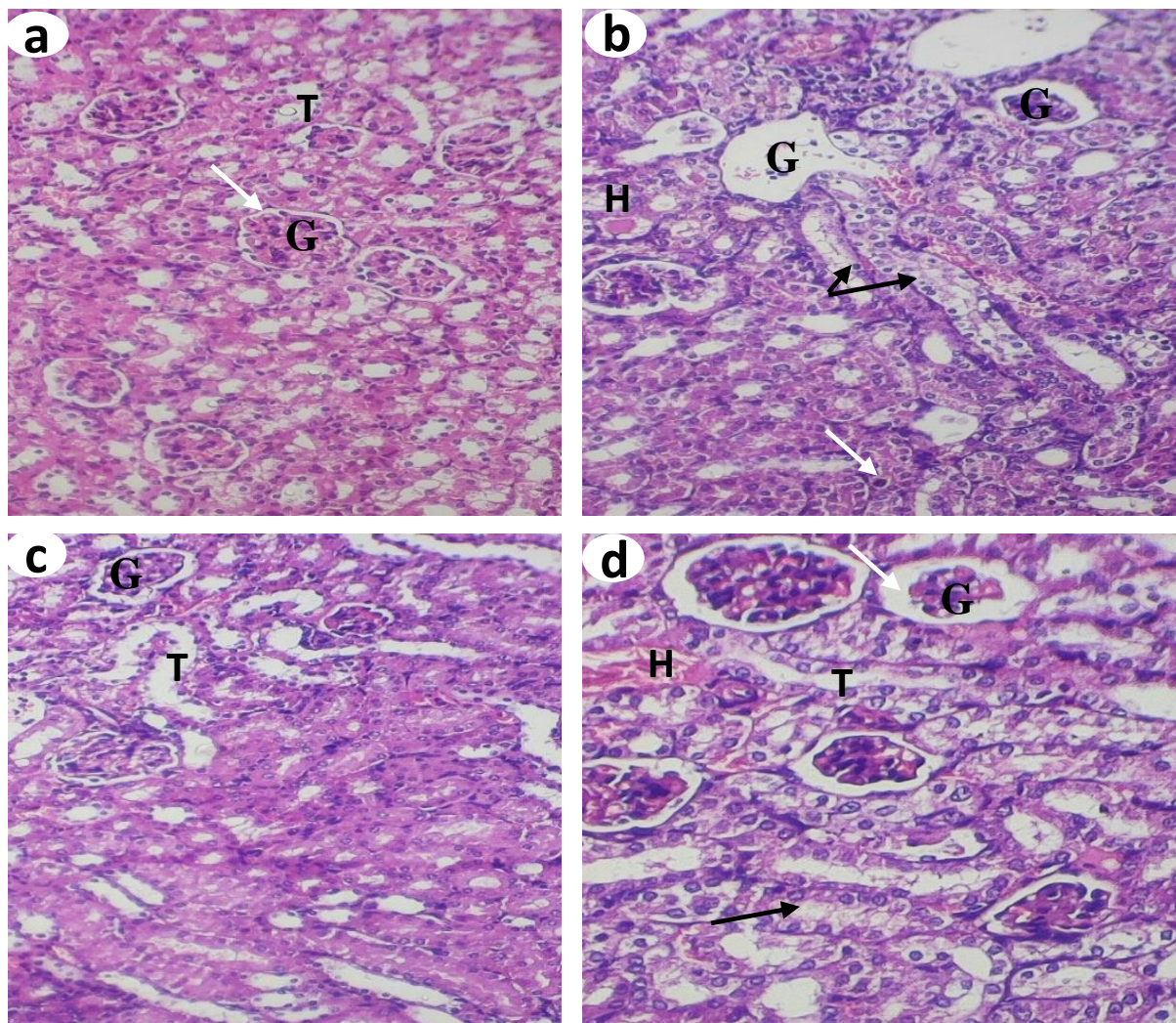
**Figure 6:** Light micrograph of liver sections in untreated and treated EAC-bearing mice. (a) Liver section of control mice showing normal architecture of hepatic lobules with polyhedral hepatocytes, with central nuclei, arranged in strands separated by blood sinusoids (bs) around the central vein (cv) with the normal arrangement of Kupffer cells (arrow). (b) EAC-treated mice showing degeneration of hepatocytes with foamy cytoplasm and binucleated hepatocytes (thick white arrow), basophilic infiltration (thin white arrow), and vacuoles (black arrow). (c) EAC/Cis treated mice showing improvement in hepatic lobules section except for central vein dilation (cv), hypertrophoid Kupffer cells (white arrow), and basophilic infiltration (black arrows). (d) EAC/RVE showing less improvement with degeneration of hepatocytes, binucleated hepatocytes (white arrow), hepatocellular necrosis (n), and basophilic infiltration (black arrow) around the central vein (cv). Hematoxylin and eosin stain,  $\times 400$ .

presence of high content of proteins, low content of lipids, and a trace amount of carbohydrates. Similarly, a previous study reported that *Aurelia aurita* revealed a high protein content and absence of carbohydrates<sup>[35]</sup>. The electrophoretic pattern (SDS-PAGE) of RVE showed the presence of six protein bands that ranged between

15 and 70 kDa. A previous study by Kawabata *et al.*<sup>[36]</sup> revealed that the SDS-PAGE of crude extract from *Atolla vanhoeffeni* and *A. wyvillei* ranged between 20-97 kDa and 14-45 kDa, respectively. Kang *et al.*<sup>[37]</sup> reported that SDS-PAGE of *Nemopilema nomurai* crude venom ranged between 20-40 kDa and 10-15 kDa.

Furthermore, Ayed *et al.*<sup>[38]</sup> reported that there was protein bands ranged between 4 and 120 kDa separated from *Pelagia noctiluca* venom. Previous studies reported that the collagen of *R. pulmo* and venom of *Acromitus flagellates* contained high

molecular weight protein varied between 105-92 and 260-3.5 kDa, respectively<sup>[39,40]</sup>. The data showed that the LD<sub>50</sub> value of RVE after 24 hours of i.p injection in mice was 1.8 g/kg b.wt. Talluri *et al.*<sup>[35]</sup> reported that LD<sub>50</sub> of *A. aurita* was 2 g/kg b.wt.



**Figure 7:** Light micrograph of kidney sections in untreated and treated EAC-bearing mice groups. (a) Kidney section of control mice showing normal structure of renal tubules (T) and glomeruli (G) surrounded with Bowman's capsule (white arrow). (b) Kidney section of EAC-bearing mice showing degeneration and atrophy in glomeruli (G), vacuolization in renal tubules (black arrows), infiltration or pyknotic cells between renal tubules (white arrow), and interstitial hemorrhages (H). (c) Kidney section of EAC bearing mice treated with Cis showing only dilation in tubules (T) and hypertrophy of glomerulus cells (G). (d) Kidney section of EAC/RVE showing tubular (T) and glomeruli (G) atrophy with dilation in Bowman's space (white arrow), interstitial hemorrhages (H), and vacuolization in renal tubules (black arrow). Hematoxylin and eosin stain,  $\times 400$ .

The cytotoxic effect of RVE was evaluated against the HepG-2 and MCF-7 cancer cells *in vitro*. The data showed that the RVE has

a cytotoxic effect on these cancer cells post 24 hours of exposure *in vitro*. A previous study showed that jellyfish extract had

anticancer activity against lung (A549) cancer cells<sup>[40]</sup>. Furthermore, Lee *et al.*<sup>[41]</sup> reported that the anticancer effect of *N. noumurai* venom against HepG-2 cancer cells. Therefore, the antitumor effect of RVE could be due to the presence of this enzyme, which can inhibit the adhesion and proliferation of cancer cells.

As compared with EAC-bearing mice, the b.wt, tumor volume, total tumor cells count, and the live tumor cells in EAC-bearing mice treated with Cis showed a significant decrease. This could be due to the potential effect of Cis as a cytotoxic and anticancer agent. Treatment of EAC-bearing mice with RVE led to significant decreases in the same previous parameters when compared with those values in non-treating EAC-bearing mice, but not much as in EAC-bearing mice that were treated with Cis. This could be due to the potential effect of RVE as an anticancer agent without toxicity. These results were in accordance with the previously published data that the b.wt and the total tumor volume were decreased due to the antitumor effect of Cis<sup>[4]</sup>. The toxic effect of the EAC tumor was confirmed by the hematological parameters. The data showed that tumor inoculation increased the total WBCs count and decreased the RBCs and platelets counts, Hb content, and Hct value in EAC-bearing mice. These findings were in agreement with a previous study that showed the major problem of tumor is myeloid suppressor and anemia<sup>[42]</sup>. Treatment of EAC-bearing mice either with Cis or RVE, decreased the total count of WBCs. In accordance with this finding, El-Naggar *et al.*<sup>[4]</sup> reported that Cis treatment elevated the total RBCs and Hb content in EAC-bearing mice. The obtained data were also in agreement with a previous study finding that natural products could provide an adjuvant beneficial effect against induced immunosuppression in mice<sup>[43]</sup>.

Serum ALT has commonly used as a specific marker of hepatocellular injuries. The progression of EAC-bearing mice led to liver dysfunctions evidenced by a significant increase in ALT activity. The elevation in

serum ALT in EAC-bearing mice may be due to the disturbance in the transport function and the leakage of the enzyme with alteration in liver permeability<sup>[44]</sup>. The cytotoxic effect of EAC led to damage of liver cells and canaculi<sup>[45]</sup>. Treatment of Cis or RVE decreased significantly the activity of serum aminotransferases in EAC-bearing mice. This could be due to the elimination of the stress caused by the hepatic tissues. These results were further evidenced by liver histopathology. The detection of kidney injury currently requires the use of conventional biomarkers of kidney function, specifically, serum urea and creatinine levels. As compared with the control groups, urea and creatinine levels were increased in EAC-bearing mice, which could be attributed to the catabolic effect of the tumor and the elevation in urea production and muscle necrosis<sup>[46]</sup>.

The histopathological investigation of liver sections of EAC-bearing mice showed the degeneration of hepatic lobules, binucleated hepatocytes, megakaryocytes, loss of cell boundaries and aggregation of basophilic inflammatory cells. However, treatment of EAC-bearing mice either with Cis or RVE ameliorated the liver architecture. A previous study reported that using natural products for treatment was able to improve the improper liver tissue architecture and eliminate its toxicity<sup>[43]</sup>. Recent studies have reported that the therapeutic dose of Cis could cause liver and kidney toxicity<sup>[4]</sup>. Interestingly, inoculation of tumors in mice causes atrophy and degeneration of glomerulus, aggregation of basophilic infiltration, and vacuolization in renal tubules. Whereas Cis and RVE improved the kidney structure, this is evidenced by the decrease in urea and creatinine levels in the current study.

In conclusion, the RVE had a cytotoxic effect against HepG-2 and MCF-7 cells *in vitro*, and showed an antitumor effect against EAC-bearing mice, but not much better than Cis did. RVE treatment also ameliorated the hepato-renal dysfunctions ensured by improving the biochemical

parameters and histopathological alterations induced in EAC-bearing mice.

#### FUNDING SOURCE DISCLOSURE

This research received no grant from any funding agency.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTIONS

WMS, SAEN, and EHH planned, conceived, and designed the study. HMA, WMS, and SAEN performed the experiments and the statistical analysis. WMS and SAEN discussed and interpreted the results. EHH performed the histopathological part and discussed its results. All the authors drafted and revised the manuscript and approved its submission.

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**How to cite this article:**

Salama, W. M.; El-Naggar, S. A.; Attia, H. M. and Hamed, E. H. (2022). The cytotoxic effect of rhizostome jellyfish venom on cancer cell lines and its antitumor activity in Ehrlich ascites-bearing mice. *Egyptian Journal of Zoology*, 78: 15-30 (DOI: 10.21608/ejz.2022.120663.1072).

## السُّمية الخلوية لسُم قناديل البحر علي خطوط خلايا سرطانية ونشاطه المضاد للورم في الفئران الحاملة لخلايا الاستسقاء الورمي البطني

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تُعتبر التأثيرات الضارة المصاحبة للعلاج الكيميائي للسرطان دافع قوي للبحث عن أدوية جديدة أكثر فاعلية. لذلك هدفت هذه الدراسة إلى تقييم الفاعلية السُّمية والمضادة للأورام لمستخلص سُم قناديل البحر "*Rhizostoma octopus*" على خطوط الخلايا السرطانية وفي الفئران الحاملة لخلايا الاستسقاء الورمي البطني، على التوالي. تم توزيع أربع مجموعات من إناث الفئران (ن=10) من سلالة "CD-1" كالتالي: المجموعة الأولى عوملت كمجموعة ضابطة. أما المجموعات الثانية حتى الرابعة فتم حقنها في التجويف البطني بخلايا الاستسقاء الورمي البطني ( $10^6 \times 1$  خلية/فأر). بعد 24 ساعة، تم حقن المجموعتين الثالثة والرابعة بعقار السيسبلاتين (2 مجم/كجم من وزن الجسم) أو بجرعة تعادل عشر الجرعة نصف المميتة من مستخلص سُم قناديل البحر (180 مجم/كجم من وزن الجسم) داخل التجويف البطني يوميا لمدة 6 أيام متتالية، على التوالي. وفي اليوم "14" من بداية التجربة تم فحص التغير في وزن الجسم، ومؤشرات الورم، والقياسات الدموية والبيوكيميائية، والتغيرات النسيجية في الكبد والكلى. وقد أظهرت النتائج أن مستخلص سُم قناديل البحر يحتوي علي كمية عالية من البروتين متضمنة ستة بروتينات، وزنها الجزيئي: 15-70 كيلو دالتون، تم قياسها بواسطة تقنية "SDS-PAGE". كما أظهرت النتائج أن قيم "IC<sub>50</sub>" لمستخلص سُم قناديل البحر على خطوط الخلايا لسرطان الكبد وسرطان الثدي كانت 808.4 و 896.4 ميكروجرام/مل، على التوالي. أما في الفئران الحاملة لخلايا الاستسقاء الورمي البطني، فقد أدى العلاج بمستخلص سُم قناديل البحر إلي تقليل التغير في وزن الجسم، وحجم الورم، وعدد الخلايا السرطانية. بالإضافة إلى ذلك، تم تحسين كل من وظائف الكبد والكلى في الفئران الحاملة لخلايا الاستسقاء الورمي البطني والمعاملة بمستخلص سُم قناديل البحر، كما يتضح ذلك من تحسن بنيتهما النسيجية.