

Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJC CBR

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH

<https://jcbr.journals.ekb.eg>

Editor-in-chief

Prof. Mohamed Labib Salem, PhD

Treatment with crude venom from the Egyptian cobra (*Naja haje*) ameliorated toxicity induced after challenge of mice with Ehrlich ascities tumor

Hosni Neweigy, Mona S. Gouda, and Mohamed S. Elnaggar



PUBLISHED BY

EACR EGYPTIAN ASSOCIATION
FOR CANCER RESEARCH

Since 2014

Treatment with crude venom from the Egyptian cobra (*Naja haje*) ameliorated toxicity induced after challenge of mice with Ehrlich ascities tumor

Hosni Neweigy¹, Mona S. Gouda², and Mohamed S. Elnaggar¹

¹Zoology department, Faculty of Science, Suez Canal University, Ismailia, Egypt

²Mansoura Children Hospital, Mansoura University, Mansoura, Egypt

ABSTRACT

Background: Cancer is one of the human mortality leading causes. While there are many tumor treatment modalities, chemotherapy remains the most effective treatment. Due to its severe side effects and developed resistance, pharmaceutical industries investigate novel antitumor strategies, especially from natural resources. **Aim:** The present work aimed to study the biochemical and histological alterations after *Naja haje* crude venom treatment to detect its antitumor efficacy. **Materials and Methods:** After experimental determination of cobra venom LD₅₀, mice were divided into three main groups, control, solid tumor and soft tumor. Control sub-groups contain saline-treated group, positive control treated with standard drug (Cisplatin), and 3 groups treated with cobra venom (1/10, 1/20 LD₅₀ and 1/30 LD₅₀). Solid tumor sub-groups included saline-treated group, cisplatin-treated group and 2 groups treated with cobra venom (1/10 and 1/20 LD₅₀). Tumor sub-groups included saline-treated group, cisplatin-treated group and 3 groups treated with cobra venom (1/10, 1/20 LD₅₀ and 1/30 LD₅₀). Serum, liver, kidney, heart, spleen, and solid tumor tissues were collected for biochemical and histopathological investigations. **Results:** The histological and biochemical results confirmed the significant cellular injury in liver. Kidney, heart and spleen, and its severity decreased by decreasing venom dose. Also, the direct anti-tumor effects of Cobra venom in both solid and soft tumors were significantly confirmed in comparing with cisplatin groups. **Conclusion:** Anti-totoxic activities of cobra venome upon tumor challenge only suggest a clinical potentiality for *Naja haje* crud venom. Further investigation should be conducted to confirm its safety and efficacy as an antitumor therapeutic agent.

Keywords: Anti-tumor effect, Biochemistry, Egyptian cobra venom, , Histology, , *Naja haje*, Toxicity

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/jcbr.2022.77445.1215

ARTICLE INFO

Article history

Submitted: June 25, 2021
Revised: December 12, 2021
Accepted: January 4, 2022

Correspondence to

Mohamed S. Elnaggar
Zoology department, Faculty of Science
Suez Canal University, Ismailia, Egypt
Tel.: 01128343043
Email: msemaenr2000@gmail.com

Copyright

©2022 Hosni Neweigy, Mona S. Gouda, Mohamed L. Salem, Mohamed S. Elnaggar. This is an Open Access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Cancer is a major health problem globally, with estimated new cases, 18.1 million and 9.6 million deaths in 2018 (Bray et al. 2018). Treatment options are radiation therapy, surgery, chemotherapy, immunotherapy, and hormonal therapy (Baskar et al. 2012). Surgery, chemotherapy, and radiotherapy provide inadequate effects or affect normal cells along with the diseased ones (Jain et al. 2012). Studies on cobra venom's effect on cancer back to several decades ago, Kurotchkin in 1935 discovered that cobra venom could destroy the Fujinami sarcoma cells in rats, limiting its

therapeutic value to direct contact between venom and tumor cells (Kurotchkin and Spies (1935). Macht In 1936 injected 105 cancer patients with a relieving pain dose of cobra venom among the patients, 23 cases showed slight relief, 30 cases showed definite relief and 38 cases showed marked relief. Only 13.3% of the patients showed doubtful or no relief (Macht 1936). A recent finding demonstrated that Moroccan cobra *Naja haje* venom exhibited anti-hepatocellular carcinoma, especially the F7 fraction of gel filtration chromatography which possesses the ability to inhibit cancer cells proliferation as the study suggested (Lafnoute et al. 2021).

Egyptian cobra (*Naja haje*) venom causes local pain, swelling, and may be associated with blistering at the bite site (Tohamy et al. 2014). The unique properties of *Naja haje* crude venom investigated for years, now recent advanced techniques give us great knowledge about the components of the crude venom and their accompanying effects, for example, *Naja haje* venom contains phospholipase 2 which may affect the cell membrane integrity (Okumu et al. 2020; Gutiérrez and Own by 2003), Mocarhagin is a cobra (snake venom metalloproteinases; SVMPs) that cleaves a glycoprotein affects platelet adhesion and neutrophil rolling and has haemagglutinin activity (Ward et al. 1996).

Also, there are Cytotoxins positively charged polypeptides that constitute about 60% of all proteins in cobra venom showed the ability to penetrate living cancer cells (human lung adenocarcinoma A549 and promyelocytic leukemia HL60) and accumulate in lysosomes (Feofanov et al. 2004). Snake α -Neurotoxin which targets muscle-type nicotinic acetylcholine receptor (nAChR), Upon binding to the nAChR, α -neurotoxins prevent the binding of the natural ligand acetylcholine (ACh) and the subsequent ACh-induced ion flow, resulting in a neuromuscular inhibition of the envenomated species (Takacs et al. 2001).

Cysteine-rich secretory proteins (CRISPs) they believed to inhibit smooth muscle contraction and block cyclic nucleotide-gated ion channels (Osipov et al. 2005). *Naja haje* cobra venom factor (CVFh) protein, can activate the complement cascade, structurally and functionally cobra venom factor resembles C3b, the activated form of C3 (von Zabern et al. 1982; and Kock et al. 2004). This complex mixture of enzymes and proteins acting together with high specificity is implicated in the pathophysiological and toxicity of *Naja haje* venom.

In this study, we are hypothesizing that *Naja haje* crud venom has a direct antitumor effect and we are aiming to test the histological and biochemical alterations on different organs accompanying the use of *Naja haje* crud venom in treating cancer on murine models.

MATERIALS AND METHODS

Mice

Female Balb/c mice aged 2–3 months were obtained from Helwan animal house of the Biological Products & Vaccines (VACSERA), Cairo Egypt, and animals were maintained in a 12-hours light /12-hours dark cycle under a suitable temperature ($20 \pm 4^\circ\text{C}$), commercial standard diet and tap water were provided. Any mouse with a palpable mass, weight loss, or change in body condition were excluded so that only healthy elderly mice were examined and all sample collection performed in the morning. All experiments were performed according to the Code of Practice for the care and use of animals for scientific purposes as per faculty of science Suez Canal University and faculty of science Tanta University.

Cell line

Ehrlich ascites carcinoma cell line (EAC) was obtained from the National Institute of Cancer, Cairo, Egypt. Fresh Ehrlich ascites carcinoma (EAC) cells were grown in mice by sequential intraperitoneal (i.p) transfer, after fifteen days EAC cells were used for experiments. The tumor cells were maintained by serial intraperitoneal transplantation of 2×10^6 cells.

Venom

Cobra venom was milked and lyophilized at the faculty of science Suez Canal University, Ismailia. We determined Cobra venom Lethal Toxic Dose LD_{50} experimentally (Meier and Theakston 1986) and who Guidelines for the Production, Control, and Regulation of Snake Antivenom Immunoglobulins 2018).

Chemicals

Commercial chemicals for investigating Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) from Human. Creatinine, and Lactate dehydrogenase (LDH) activity kits from Biodiagnostic.

Experimental design

Lethality was determined by intraperitoneal (i.p) injection of different doses of the venom in 0.5 ml saline to 8 male Balb/c mice, the survival time recorded and LD_{50} calculated to be 0.568 mg/kg (Meier and Theakston 1986). Mice were

separated into three general groups (control-treated, solid tumor (subcutaneous tumor), and soft tumor (Ehrlich ascites tumor). Control groups contain 5 sub-groups (healthy control group treated with saline, control group treated with cisplatin, control group treated with 1/10 LD₅₀, control group treated with dose 1/20 LD₅₀, and control group treated with dose 1/30 LD₅₀). Solid tumor (subcutaneous tumor) groups contain 4 subgroups (solid tumor (S.T. treated with saline), solid tumor Cis (S.T. Cis, treated with cisplatin), solid tumor 1/10 (S.T. 1/10 treated with a crud venom dose 1/10 LD₅₀), and solid tumor 1/20 (S.T. 1/20 treated with dose 1/20 LD₅₀). Soft tumor (ascites tumor (Asc) groups contain 5 sub-groups (ascites tumor (Asc treated with saline), (ascites tumor (Asc + Cis) treated with cisplatin), (ascites tumor (Asc + 1/10) treated with a crud venom dose 1/10 LD₅₀), (ascites tumor (Asc + 1/20) treated with a crud venom dose 1/20 LD₅₀) and soft tumor (Asc + 1/30) group treated with a crud venom dose 1/30 LD₅₀). Each mouse was injected with 0.5 ml containing the desired dose of venom 1/10 LD₅₀, 1/20 LD₅₀, 1/30 LD₅₀, or 40 U of Cisplatin two times onset (28 days for solid tumor and 10 days for soft tumor) and after 5 days then sacrificed at day 10. The solid tumor model was produced by inoculating the Ehrlich ascites carcinoma cell line for 28 days by injecting about 2×10^6 cells subcutaneously. Soft (Ascites) tumor model produced by inoculating Ehrlich ascites carcinoma cell line for 10 days by injecting about 1.5×10^6 cells intraperitoneal. The following organs were collected: heart, kidneys, liver, and spleen also solid tumor, and ascites tumor. Tissues were fixed in 10% buffered formalin, and then processed in paraffin; 5µm sections were prepared and stained with H&E. The slides were then studied under the light microscope. Ascites tumor volume determined by a graded falcon tube while solid tumor measured with a caliper and tumor volume was calculated using the formula $V \text{ (mm}^3\text{)} = a \times b^2/2$, where V (mm³) is the tumor volume in mm³, a=length, b=width of the tumors (Ikitimur-Armutak El et al. 2016).

Statistical analysis

Statistical analysis was performed on SPSS v 14 software. Data were presented as mean ± SD. Statistical significance was analyzed

independent T-test comparing two groups. $P \leq 0.05$ was considered significant.

RESULTS

Before the end of our experiment some animals died (Table 1). Yet we couldn't detect any tumor mass in one animal treated with venom dose 1/20 and cisplatin, also we could not detect any ascites tumor in one animal treated with venom dose 1/30 there is no data from those animals.

Biochemical study

All groups have been investigated for the following biochemical factors (ALT, AST, LDH, and Creatinine) and solid tumor volume, results in mean shown in Table 1. Compared to healthy control ALT enzyme activity (Figure 1A) increased significantly in the control group treated with cisplatin ($P=0.050$), solid tumor group treated with 1/10 LD₅₀ of crude venom ($P=0.002$), solid tumor treated with cisplatin ($P=0.000$), soft ascites tumor control group ($P=0.019$) and in soft ascites tumor treated with 1/20 LD₅₀ venom dose ($P=0.007$) also there was a significant decrease in ALT activity in Ascites tumor treated with 1/30 LD₅₀ venom ($P=0.007$). On the other hand, ALT increased in the solid tumor group treated with 1/10, 1/20 doses and cisplatin ($P=0.001$, 0.260 and 0.000 when compared to the solid tumor control group, also it decreased in ascites groups treated with cisplatin, 1/20 and 1/30 ($P=0.176$, 0.653 and 0.090) when compared to the ascites control group.

AST Enzyme activity (Figure 1A) significantly increased in the control group treated with 1/10 LD₅₀, solid tumor groups treated with 1/10 LD₅₀, 1/20 LD₅₀, cisplatin, and control solid tumor group ($P=0.000$, 0.000, 0.002, 0.014, and 0.002) also, it increased in soft ascites tumor groups, treated with 1/20, 1/30, cisplatin and ascites tumor control group ($P=0.000$, 0.065, 0.000 and 0.000) compared to normal control. On the other hand, AST increased in the soft (ascites) tumor group treated with cisplatin and 1/20 LD₅₀ venom dose ($P=0.010$ and 0.107), while it decreased in ascites treated with 1/30 LD₅₀ ($P=0.046$) when compared to the ascites control group. Creatinine increased with no statistical significance (Figure 1B) in all studied groups except for soft tumors treated with 1/20 LD₅₀

venom dose it increased with statistical significance ($P= 0.054$) when compared to healthy control. Creatinine increased in solid tumors treated with cisplatin, 1/10 and 1/20 compared to the solid tumor control group. On the other hand, it increased in ascites tumors treated with cisplatin, 1/20 and 1/30 LD₅₀ compared to the ascites tumor control group. Lactate dehydrogenase (LDH) increased (Figure 1C) with no statistical significance in all control groups, it also increased significantly in solid tumors treated with cisplatin, soft tumor control group (Ascites control), and ascites tumor treated with 1/20 LD₅₀ ($P= 0.000, 0.000, 0.000$) when compared to healthy control. LDH increased in solid tumor treated with cisplatin ($P=0.001$) compared to the solid tumor control group. On the other hand, it increased in ascites tumors treated with cisplatin and 1/20 LD₅₀ ($p= 0.002$ and 0.005) and decreased significantly on ascites tumor treated with 1/30 LD₅₀ ($P= 0.006$) when compared to the ascites tumor control group.

Histological study

Compared to the healthy control group the liver of the control group treated with 1/10 LD₅₀ of venom dose showed hepatic vacuolation associated with foci of mononuclear cells aggregation and a few apoptotic cells, also control group treated with 1/20 LD₅₀ showed mild hepatic vacuolation and few apoptotic cells while control group treated with 1/30 LD₅₀ of venom dose showed granular hepatic degeneration and mild perivascular mononuclear cells, on the other hand, cisplatin showed inflammatory cells infiltration granular hepatocytes degeneration and single-cell hepatic necrosis. Among solid tumor groups (subcutaneous tumor mass) Liver of control positive animal showed vacuolation of hepatocytes, mice treated with 1/10 of LD₅₀ of venom dose showed a marked increase in the apoptotic hepatocytes, while mice treated with 1/20 of LD₅₀ of venom dose showed hepatic vacuolation and a few apoptotic hepatocytes. Mice treated with cisplatin showed apoptotic hepatocytes and necrotic foci associated with mononuclear cells aggregation. Among soft tumor groups, the Liver of the control positive animal showed focal aggregation of the mononuclear cells associated with numerous

apoptotic hepatocytes. Liver of diseased animals treated with 1/20 LD₅₀ of venom dose showed few apoptotic hepatocytes. Liver of diseased animals treated with 1/30 LD₅₀ of venom dose showed few degenerated hepatic cells. The liver of diseased animals treated with cisplatin showed numerous apoptotic hepatocytes.

Compared to the healthy control group the toxic effect of venom on kidney decreased by decreasing venom dose, Kidney of control group treated with 1/10 LD₅₀ of venom dose showing congestion of the renal blood capillaries and marked degenerative changes within the lining epithelium of the renal tubules, in the control group treated with 1/20 LD₅₀ of venom dose showed a moderate degree of tubular epithelium degeneration, and in the control group treated with 1/30 LD₅₀ of venom, dose showed normal tubular epithelium lining, while in animal treated with cisplatin the renal tubules showed vacuolar degeneration. Among solid tumor groups, the Kidney of the control positive animals showed mild degenerative changes within the lining epithelium of the renal tubules. Kidney of solid tumor group treated with 1/10 LD₅₀ of venom dose showed marked degenerative changes within the lining epithelium of the medullary renal tubules, also in solid tumor group treated with 1/20 LD₅₀ of venom dose showed mild tubular epithelium degeneration. On the other hand in solid tumor group treated with cisplatin showed necrotic changes within the renal tubules and vacuolar degeneration of the renal tubules. Among soft tumor groups, the Kidney of control positive animals showed congestion of the glomerular tuft and marked degenerative changes within the lining epithelium of the renal tubules. Soft tumor groups treated with 1/20 LD₅₀ of venom Showed vacuolation of the lining epithelium of the renal tubules while Soft tumor groups treated with 1/30 LD₅₀ of venom showed a mild degree of tubular epithelium degeneration. On the other hand, the soft tumor group treated with cisplatin showed congestion of the renal capillaries and marked degenerative changes within the renal tubules. Compared to the healthy control group the Heart of group treated with 1/10 LD₅₀ of venom showed sarcoplasmic eosinophilia and marked myolysis

of the myocardial fibers, Heart of normal animal treated with 1/20 LD₅₀ showed mild sarcoplasmic eosinophilia, also control group treated with cisplatin showed myolysis and sarcoplasmic eosinophilia on the other hand normal animal treated with 1/30 LD₅₀ showed normal myocardial fibers Heart. Among solid tumor groups, Heart of control positive animals showed mild myolysis of the myocardial fibers. While the heart of mice treated with 1/10 LD₅₀ of venom showed marked myolysis of the myocardial fibers, animals treated with 1/20 of LD₅₀ showed sarcoplasmic eosinophilia and a mild degree of myolysis, on the other hand, animals treated with cisplatin showed myocarditis indicates infiltration of mononuclear inflammatory cells. Among soft tumor groups, Heart of the control positive animal showed mild to moderate degree of myocardial degeneration, mice treated with 1/20 LD₅₀ of venom showed myocardial degeneration associated with myolysis, animals treated with 1/30 LD₅₀ showed sarcoplasmic eosinophilia, and a mild degree of myolysis and Heart of diseased animal treated with cisplatin showing myocardial degeneration.

Compared to the healthy control group the Spleen of the control group treated with 1/10 LD₅₀ of venom showed a mild degree of lymphoid depletion, also control group treated with 1/20 LD₅₀ showed a mild degree of lymphoid follicle depletion, and the control group treated with 1/30 LD₅₀ showed a mild degree of lymphoid follicle depletion while control group treated with cisplatin showed moderate degree lymphoid follicle depletion. Among solid tumor groups, the Spleen of control positive animals showed mild lymphoid depletion, animals treated with 1/10 LD₅₀ showed lymphoid depletion indicates a decrease in the germinal lymphocytes also animals treated with 1/20 LD₅₀ showed a mild degree of lymphoid follicle depletion and animals treated with cisplatin showed a mild lymphoid follicle depletion. Among soft tumor groups, the spleen of control positive animals showed marked lymphoid depletion. Animals treated with 1/20 LD₅₀ showed normal lymphoid follicle, animals treated with 1/30 LD₅₀ showed a mild degree of lymphoid follicle

depletion also animals treated with cisplatin showed a mild lymphoid follicle depletion. The Subcutaneous tumor mass examination (Figure 2A) of control positive animals showed increased proliferated neoplastic Ehrlich cells with limited areas of necrosis, animals treated with 1/10 LD₅₀ venom dose. Figure 2B showed marked increased necrotic cells and decreased neoplastic Ehrlich cells within the mass, also animals treated with 1/20 LD₅₀ venom dose. Figure 2C showed decreased proliferated neoplastic Ehrlich cells with increased necrotic areas, and animals treated with cisplatin. Figure 2D showed marked decrease neoplastic cells with increased necrotic areas. Solid Tumor mass (Figure 1D) decreased in all treated groups 1/10, 1/20, and cisplatin (P= 0.707, 0.146 and 0.822) compared to solid tumor non treated group, also soft tumor mass (Figure 1E) decreased in all treated groups 1/20, 1/30 and cisplatin (P= 0.940, 0.619 and 0.273) compared to ascites tumor non treated group

DISCUSSION

Liver enzymes are important in assessing and monitoring liver inflammation and necrosis which result in the release of both enzymes (ALT and AST) in circulation due to increased permeability of the cell membrane or breakdown of the cells (Abdel Moneim et al. 2013). Our results demonstrated that the liver enzyme ALT decreased in all healthy controlled groups treated with venom doses 1/10, 1/20, 1/30 LD₅₀ with no statistical differences compared to healthy control groups these results agreed with previous results of which reported decreased levels of serum ALT in a dose of 1/2 LD₅₀ of *Naja haje* venom on mice after 6 days of envenomation (Tohamy et al. 2014), and disagree with a previous study that recorded a significant increase in ALT in rats after 24 hours envenomation (Shaban et al. 2003) and another study demonstrated that ALT levels increases in subcutaneously envenomed animals after 3 days (Lamiaa et al. 2018). Also, our results showed that AST increased in all control groups treated with venom doses 1/10, 1/20, 1/30 these results agreed with previous studies (Tohamy et al. 2014; Shaban et al. 2003 and Lamiaa et al. 2018).

Table 1. Number of mice for each group and biochemical results (ALT, AST, creatinine, and LDH) shown in mean also tumor volume.

	Number		ALT U/L	AST U/L	Creatinine mg/dl	LDH U/L	Tumor volume (mm ³)
	Total	Died					
Control negative	8	0	49.6	176.2	0.4	3316.8	-
Cis control	9	0	54.2	191.4	0.5	5024.4	-
1\10 control	9	1	39.3	203.5	0.5	5209.0	-
1\20 control	9	1	48.4	210.9	0.5	4797.0	-
1\30 control	7	0	37.5	166.5	0.5	4678.0	-
G1 S. T + cis	9	1	72.3	278.9	0.5	10318.0	0.5
G2 S. T +1/10	9	0	81.2	326.9	0.6	6646.5	0.4
G3 S. T +1/20	9	0	69.5	309.1	0.7	9064.0	0.4
G4 S. T	9	1	49.1	401.1	0.5	7435.0	0.9
Ascites control	9	3	61.4	351.6	0.5	3341.7	3.9
Asc + Cis	9	1	53.9	680.2	0.6	13080.8	3.1
Asc + 1/10	9	8	-	-	-	-	-
Asc + 1/20	9	3	52.2	447.2	0.5	10435.2	2.7
Asc + 1/30	7	2	42.8	236.5	0.5	6293.3	2.9

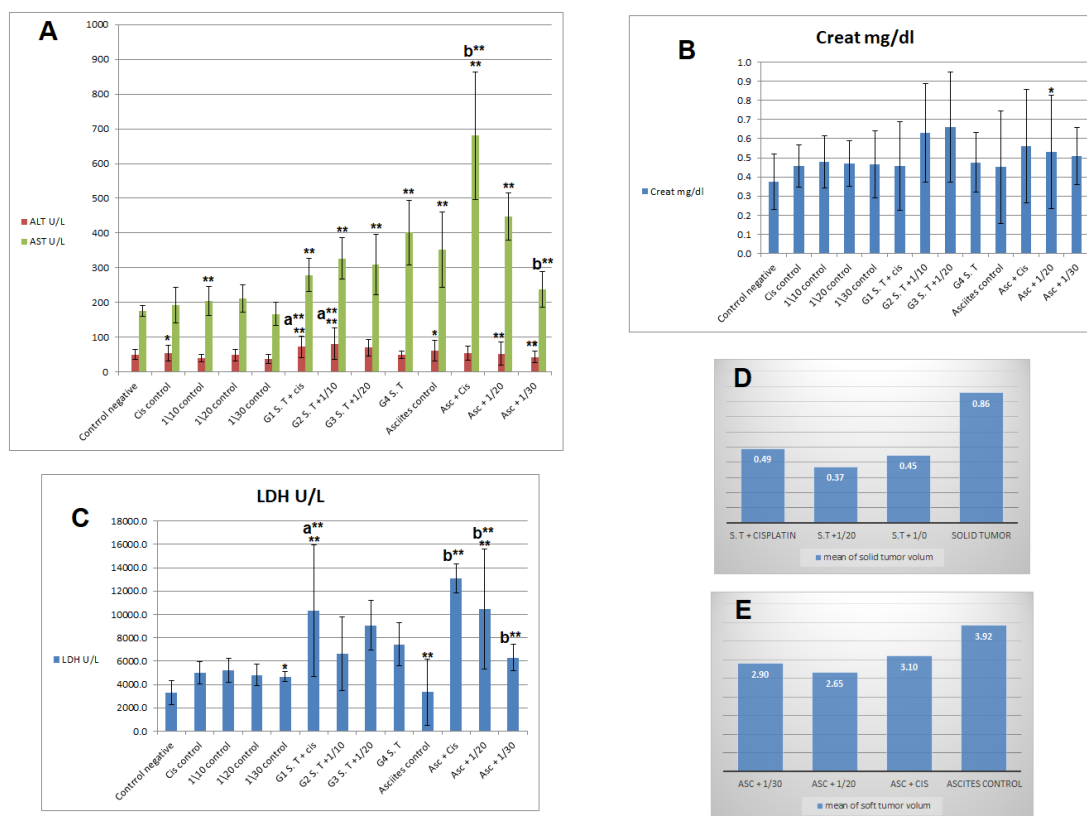


Figure 1. A) liver enzymes (ALT, AST) activity for each group, statistical significance shown on bars (* $P \leq 0.05$, ** $P \leq 0.01$) when compared to healthy control group (a** = $P \leq 0.01$) when compared to solid tumor control group (b** = $P \leq 0.01$) when compared to soft tumor (Ascites) control group. B) Creatinine levels in each group C) Lactate dehydrogenase (LDH) activity for each group D) Mean of solid tumor volume for each group E) Mean of soft tumor volume for each group

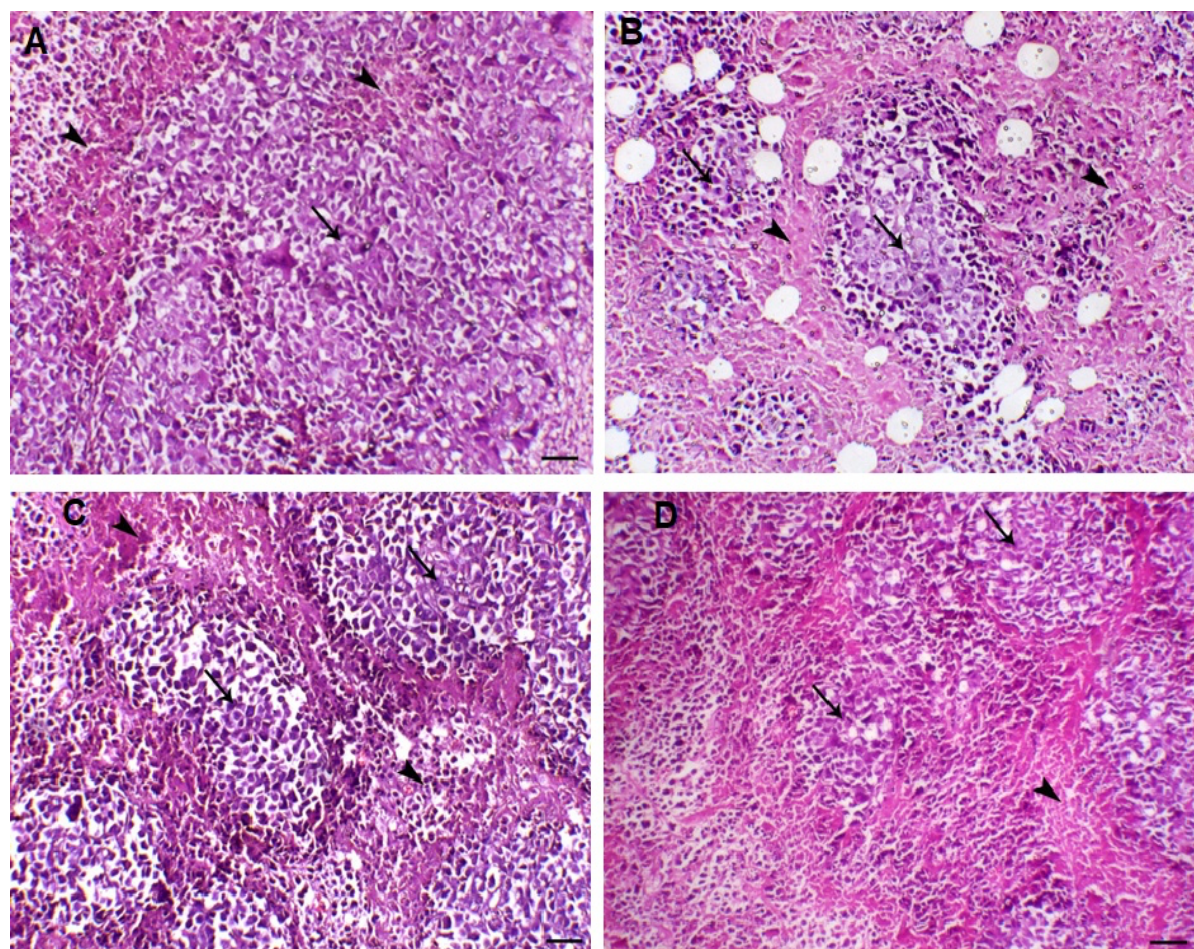


Figure 2. A) Subcutaneous tumor mass of control positive animal showing increase the proliferated neoplastic Ehrlich's cells (arrow) with limited areas of necrosis (arrowhead). B) Subcutaneous tumor mass of an animal treated with 1/10 LD₅₀ showing marked increase the necrosis (arrowhead) and decrease neoplastic Ehrlich's cells (arrow) within the mass. C) Subcutaneous tumor mass of an animal treated with 1/20 LD₅₀ showing a decrease in the proliferated neoplastic Ehrlich's cells (arrows) with an increase in the areas of necrosis (arrowheads). D) Subcutaneous tumor mass of an animal treated with cisplatin showing marked decrease of the neoplastic cells (arrows) with increase the areas of necrosis (arrowhead), H&E, X200, bar= 80 μ m.

All this data was confirmed by our results of liver histology, control group treated with 1/10, 1/20 and 1/30 LD₅₀ of venom doses showed tissue alterations and cellular hepatic toxicity these findings agreed with the previous study (Tohamy et al. 2014), also the toxic effect decrease with decreasing venom dose.

Our results demonstrated that creatinine levels increased in healthy control animals envenomed with 1/10, 1/20 LD₅₀, and normal kidney histology for the control group treated with 1/30 LD₅₀ these results agreed with previous findings (Tohamy et al. 2014; Shaban et al. 2003 and Lamiaa et al. 2018) these results confirmed by our histological findings for kidney showing direct toxicity on kidney cells (degenerated cells) affecting their function and tissue injury (congestion and inflammation),

these findings agreed with previous studies (Tohamy et al. 2014 and Shaban et al. 2003).

Lactate dehydrogenase (LDH) plays an important role in cancer metabolism by converting pyruvate to lactate and vice versa (Valvona et al. 2016), Cancer cells convert glucose to lactate by the process of aerobic glycolysis called the (Warburg effect) (Vander et al. 2009), Our data revealed that Lactate dehydrogenase (LDH) increased when mice treated with *Naja haje* crude venom doses 1/10, 1/20 and 1/30 these data agree with previous studies demonstrated this effect of *Naja haje* venom (Shaban et al. 2003; Lamiaa et al. 2018 and Mohamed et al. 1981.)

Compared to the healthy control group treated with doses 1/10 and 1/20 showed heart and

spleen tissue alterations These results agree with previous studies (Shaban et al. 2003; Lamiaa et al. 2018 and Rania et al. 2015) reported degenerated muscle fibers with loss of striations on the heart and reduced white pulps with ill-defined outlines of the spleen (Shaban et al. 2003 and Rania et al. 2015) those effects going milder by decreasing venom dose.

Many studies (Tohamy et al. 2014; Shaban et al. 2003 and Lamiaa et al. 2018) regarded the histological changes accompanying *Naja haje* crud envenomation to The phospholipase, which may cause the hepatic vacillation and degeneration as it affects cell membrane permeability it alters the ionic gradients by decreasing Na⁺/K⁺ ATPase activities, as suggested by Chethankumar and Srinivas (Chethankumar et al. 2008). Although it triggers many mechanisms by forming multimeric complexes with other venom components (Doley and Kini (2009). *Naja haje* venom phospholipase concentration is still low compared to other cobra species (Tan et al. 2019). So we can explain our findings that other factors in *Naja haje* venom can alter these histological changes, also the toxic effect can be reduced for therapeutic purposes by refining the venom complex to specific working therapeutic proteins or enzymes.

liver enzymes ALT and AST increased in the control group treated with cisplatin, also creatinine and LDH activity these findings agreed with a study that found elevation of ALT three folds and AST five-folds after 24-hour cisplatin injection (Lu and Cederbaum (2005), another study found a significant elevation of ALT, AST, and creatinine on treated mice (Athira et al. 2016) also a study record increased LDH when treating healthy control animal with cisplatin (Xing et al. 2019).

Our histological studies confirm our lab findings on animals treated with cisplatin, these results agreed with previous studies (Lu and Cederbaum (2005) on the liver but with no observed necrosis, and (Athira et al. 2016) on liver and kidney. Our findings on spleen agree with study showed changes in spleen architecture and hemosiderin deposition (Banerjee et al. 2018), also our findings on heart agree with (Topal et al. 2018) which observed

heart necrosis, dilated/congested blood vessels, hemorrhage, polymorphonuclear leukocyte, edema, and cells with pyknotic nuclei in the cisplatin-treated group.

AST, ALT, and creatinine increased in both positive tumor control groups (solid and soft tumor) these results agreed with previous studies (Kapoor et al. 2014 and Saad et al. 2017) these biochemical results were confirmed by our histological results of Liver and kidney and agree with other studies (Kapoor et al. 2014 and Salem et al. 2011).

Our findings agreed with previous work (Mishra et al. 2018) reported increased liver enzymes and kidney function, also liver degeneration and inflammation and inflammatory cells in the interstitial space of the kidney they also recorded heart fibrosis, marked atrophy, and contractile dysfunction in Subcutaneous Ehrlich Ascites Carcinoma mice model, we couldn't confirm their findings of spleen cellular proliferation, also we agree with the previous study (Hashem et al. 2020) reported renal epithelial vacuolation, glomerular tuft shrinkage and hyaline casts inside the lumina of renal tubules they also reported liver fatty changes.

Also, LDH increased in all solid tumor groups and soft tumor groups, these results agree with the previous study demonstrated that (LDH) increased in solid tumor-bearing mice (Frajacomo et al. 2016). Also, it increased in the solid tumor treated with cisplatin and 1/20 compared to solid tumor control, and soft tumor animals treated with cisplatin and 1/20 compared to soft tumor control these findings agree with the previous study of increasing LDH activity for cisplatin treating Ascites Dalton's lymphoma with cisplatin (Prasad and Anirudha (1999), but decreased in solid tumor treated with 1/10 LD₅₀ compared to solid tumor group and soft tumor treated with 1/30 compared to ascites soft tumor control group as in many studies may be related to good prognosis and survival (Liu et al. 2019 and Petrelli et al. 2015).

Our results for the Liver and kidney of diseased animal soft tumor group treated with cisplatin agree with a study (Hashem et al. 2020) Reported increased liver enzymes and creatinine also markedly dilated and congested central vein with chronic inflammatory cells

between hepatic cells, kidney showed congested vascular spaces with an extension of hemorrhage into the glomeruli.

Compared to solid tumor non treated group both venom doses 1/10 and 1/20 LD₅₀ showed the increased necrotic area and decreased proliferated neoplastic Ehrlich's cells with decreased tumor size this showed the direct anti-tumor effect of Egyptian cobra venom, previous studies on Egyptian cobra reported a similar effect in vitro on prostate cancer cell line (Mohamed AA 2003), and head, neck squamous cell carcinoma (El-Ghani and Eman MA (2020). Also, animals treated with cisplatin showed a marked decrease of the neoplastic cells with increased areas of necrosis these findings may go with a previous study that demonstrated a few necrotic tumor cells all over the area of the tumor at the subcutaneous tissue single cisplatin dose on day 12 of the subcutaneous occultation of Ehrlich cell line (Ola et al. 2018).

CONCLUSION

From our results, we can conclude, that cancer has toxic nature that contributes to its pathogenesis not just affecting the tumor localized area but also intoxicating different body organs leading to multiple pathogenic effects in these organs affecting their normal physiology and finally may lead to death. *Naja haje* venom has a direct anti-tumor effect further investigation on the venom components that target cancer cells is still needed, the venom toxicity can be reduced by reducing the dose and can be compared to current cisplatin chemotherapy.

ABBREVIATION

- Asc: Ascites
- LD₅₀: Lethal Toxic Dose 50%
- Cis: Cisplatin
- SVMs: Snake venom metalloproteinases
- nAChR: Nicotinic acetylcholine receptor
- ACh: Acetylcholine
- CRISPs: Cysteine-rich secretory proteins
- CVFh: Cobra venom factor
- i.p: Intraperitoneal
- EAC: Ehrlich ascites carcinoma cell line
- AST: Aspartate aminotransferase
- ALT: Alanine aminotransferase
- LDH: Lactate dehydrogenase

ACKNOWLEDGMENT

The authors would like to thank Walied Sobhy Abdo, Department of Pathology, Kafr-elsheikh University; for reviewing our histology work. Also, I would like to thank Ali M. Abou-Elezz PhD Student, Physiology Faculty of Science Mansoura University; for his sincere help in animal housing, injecting mice, dissection, and sampling.

References

- Abdel Moneim AE, Othman MS, Mohmoud SM, El-Deib KM. 2013. Pomegranate Peel Attenuates Aluminum-Induced Hepatorenal Toxicity. *Toxicology Mechanisms and Methods*. 23(8): 624–33. <https://doi.org/10.3109/15376516.2013.823634>.
- Athira KV, Madhana RM, Kasala ER, Samudrala PK, Lahkar M, Gogoi R. 2016. Morin Hydrate Mitigates Cisplatin-Induced Renal and Hepatic Injury by Impeding Oxidative/Nitrosative Stress and Inflammation in Mice. *Journal of Biochemical and Molecular Toxicology*. 30, 12: 571–79.
- Banerjee S, Sinha K, Chowdhury S, Sil PC. 2018. Unfolding the Mechanism of Cisplatin Induced Pathophysiology in Spleen and Its Amelioration by Carnosine. *ChemBiol Interact*. 279: 159–170.
- Baskar R, Kuo AL, Richard Y, Kheng-Wei Y. 2012. Cancer and Radiation Therapy: Current Advances and Future Directions. *International Journal of Medical Sciences*. 9(3): 193–99.
- Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J for Clin*.68: 394-424. doi: 10.3322/caac.21492
- Chethankumar M, Leela S. 2008. Gangliosides as Potential Inhibitors of NajaNaja Venom PLA2 (NV-PLA2) Induced Human Erythrocyte Membrane Damage. *African Journal of Biochemistry Research*. 2(1): 008–014.
- DoleyR, Kini RM. 2009. Protein Complexes in Snake Venom. *Cellular and Molecular Life Sciences*. 66(17): 2851–71.
- El-Ghani SF, Eman MA. 2020. A Comparative Study on Anticancer Effect of Crude Venoms of the Egyptian Naja-Haje and Viper Cerastescerastes on Head and Neck Squamous Cell Carcinoma (In Vitro Study). *Egyptian Dental Journal*. 66(1): 237–246.
- FeofanovAV, Sharonov GV, Dubinnyi MA, Astapova MV, Kudelina IA, Dubovskii PV, Rodionov DI,

- Utkin YN, Arseniev AS. 2004. Comparative Study of Structure and Activity of Cytotoxins from Venom of the Cobras *NajaOxiana*, *NajaKaouthia*, and *Naja haje*. *Biochemistry. Biokhimiia*. 69(10): 1148–57.
- Frajacomo FT, de Souza Padilha C, Marinello PC, Guarnier FA, Cecchini R, Duarte JA, Deminice R. 2016. Solid Ehrlich Carcinoma Reproduces Functional and Biological Characteristics of Cancer Cachexia. *Life Sciences*. 162: 47–53.
- Gutiérrez JM, Ownby CL. 2003. Skeletal Muscle Degeneration Induced by Venom Phospholipases A2: Insights into the Mechanisms of Local and Systemic Myotoxicity. *Toxicon: Official Journal of the International Society on Toxinology*. 42(8): 915–31.
- Hashem MA, Mahmoud EA, Abd-Allah NA. 2020. Alterations in Hematological and Biochemical Parameters and DNA Status in Mice Bearing Ehrlich Ascites Carcinoma Cells and Treated with Cisplatin and Cyclophosphamide. *Comparative Clinical Pathology*. 29: 517–24.
- Ikitimur-ArmutakEI, Ulukaya E, Gurel-Gurevin E, Yaylim I, Isbilen-Basok B, Sennazli G, Yuzbasioglu-Ozturk G, Sonmez K, Celik F, Kucukhuseyin O, Korkmaz G, Yilmaz VT, Zeybek SU. 2016. Apoptosis-inducing Effect of a Palladium(II) Complex-[PdCl(terpy)](sac).2H₂O on Ehrlich Ascites Carcinoma (EAC) in Mice. *In Vivo*. 30(4):457-464.
- Jain D, Sudhir Kumar. 2012. Snake Venom: A Potent Anticancer Agent. *Asian Pacific Journal of Cancer Prevention*. 13(10): 4855–60.
- Kapoor R, Gundpatil DB, Somani BL, Saha TK, Bandyopadhyay S, Misra P. 2014. Anticancer Effect of DL-Glyceraldehyde and 2-Deoxyglucose in Ehrlich Ascites Carcinoma Bearing Mice and Their Effect on Liver, Kidney and Haematological Parameters. *Indian Journal of Clinical Biochemistry*. 29(2): 213–20.
- KockMA, Hew BE, Bammert H, Fritzing DC, Vogel CW. 2004. Structure and Function of Recombinant Cobra Venom Factor. *J Biol Chem*. 279(29):30836-43. doi: 10.1074/jbc.M403196200.
- Kurotchkin T J, Spies JW. 1935. Effects of Cobra Venom on the Fujinami Rat Sarcoma. *Proceedings of the Society for Experimental Biology and Medicine*. 32(9): 1408–10.
- Lafnounce A, Lee S-Y, Heo, J-Y, Gourja I, Darkaoui B, Abdelkafi-Koubaa Z, et al. 2021. Anti-cancer effect of Moroccan cobra *Naja haje* venom and its fractions against hepatocellular carcinoma in 3D cell culture. *Toxins*, 13(6), 402. doi:10.3390/toxins13060402
- Lamiaa SE, Osama AA, Ola NS, Dyaa MF, Alaa GM Naglaa ER. 2018. Physiological Effects of Egyptian Cobra Venom on Albino Mice and Its Antibacterial Effect. *Int J Recent Sci Res*. 9(5), pp. 26997-27007.
- Liu D, Wang D, Wu C, Zhang L, Mei Q, Hu G, Long G, Sun W. 2019. Prognostic Significance of Serum Lactate Dehydrogenase in Patients with Breast Cancer: A Meta-Analysis. *Cancer Management and Research*. 11: 3611–19.
- Lu Y, Cederbaum AI. 2005. Cisplatin-Induced Hepatotoxicity Is Enhanced by Elevated Expression of Cytochrome P450 2E1. *Toxicological Sciences*. 89(2):515-23.
- Macht DI. 1936 Experimental and Clinical Study of Cobra Venom as an Analgesic. *Proceedings of the National Academy of Sciences of the United States of America*. 22(1): 61–71.
- Meier J, Theakston RD. 1986. Approximate LD₅₀ Determinations of Snake Venoms Using Eight to Ten Experimental Animals. *Toxicon: Official Journal of the International Society on Toxinology*. 24(4): 395–401.
- Mishra S, Tamta AK, Sarikhani M, Desingu PA, Kizkekra SM, Pandit AS, Kumar S, Khan D, Raghavan SC, Sundaresan NR. 2018. Subcutaneous Ehrlich Ascites Carcinoma Mice Model for Studying Cancer-Induced Cardiomyopathy. *Scientific Reports*. 8(1): 5599.
- Mohamed AA. 2003. In Vitro Anticancer Effect of Scorpion *LeiurusQuinquestriatus* and Egyptian Cobra Venom on Human Breast and Prostate Cancer Cell Lines. *Journal of Medical Sciences*. 3(1):66-86.
- Mohamed AH, Fouad S, El-Aasar S, Salem AM, Abdel-Aal A, Hassan AA, Zahran F, Abbas N. 1981. Effects of Several Snake Venoms on Serum and Tissue Transaminases, Alkaline Phosphatase and Lactate Dehydrogenase. *Toxicon*. 19(5): 605–9.
- OkumuMO, Mbaria JM, Gikunju JK, Mbuthia PG, Madadi VO, Ochola FO. 2020. Enzymatic activity and brine shrimp lethality of venom from the large brown spitting cobra (*Najaashei*) and its neutralization by antivenom. *BMC Res Notes*. 13(1):325. doi: 10.1186/s13104-020-05167-2.
- Ola SM, Noha AE, Ahmad MS, Nehal MA. 2018. Atorvastatin ameliorates cisplatin induced oxidative stress in Ehrlich Ascites solid tumor-bearing mice: a prospective case control study. *Al-Azhar Medical Journal*. 47(1): 97–107.
- OsipovAV, Levashov MY, Tsetlin VI, Utkin YN. 2005. Cobra Venom Contains a Pool of Cysteine-Rich Secretory Proteins. *BiochemBiophys Res Commun*. 328(1):177-82. doi: 10.1016/j.bbrc.2004.12.154.
- PetrelliF, Cabiddu M, Coinu A, Borgonovo K, Ghilardi M, Lonati V, Barni S. 2015. Prognostic Role of

- Lactate Dehydrogenase in Solid Tumors: A Systematic Review and Meta-Analysis of 76 Studies. *Acta Oncologica*. 54(7): 961–70.
- Prasad SB, Anirudha G. 1999. Effect of Cisplatin on the Lactate Dehydrogenase Activity and Its Isozyme Pattern in Dalton's Lymphoma Bearing Mice. *Cytologia*. 64(3): 259–267.
- Rania HA, Abdelaziz EI. 2015. Effects Of Egyptian Cobra (*Naja haje*) Venom On Post Mortem Changes And Some Biochemical Parameters In Rats. *IJSR - International Journal of Scientific Research(IJSR)*. 4(1)
- Saad EA, Hassanien MM, El-Mezayen HA, Elmenawy NM. 2017. Regression of Murine Ehrlich Ascites Carcinoma Using Synthesized Cobalt Complex †The Authors Declare No Competing Interests." *MedChemComm*. 8(5): 1103–1111. Salem FS, Badr MO, Neamat-Allah AN. 2011. Biochemical and Pathological Studies on the Effects of Levamisole and Chlorambucil on Ehrlich Ascites Carcinoma-Bearing Mice. *Veterinarialtaliana*. 47(1): 89–95
- Shaban EA, Hafez MN. 2003. Ability of Gamma-Irradiated Polyvalent Antivenin to Neutralize the Toxicity of the Egyptian Cobra (*Naja haje*) Venom. *Egypt. J. Hosp. Med*. 13: 135-152.
- Takacs Z, Wilhelmsen KC, Sorota S. 2001. Snake Alpha-Neurotoxin Binding Site on the Egyptian Cobra (*Naja haje*) Nicotinic Acetylcholine Receptor Is Conserved. *Molecular biology and evolution*. 18(9):1800-9. doi: 10.1093/oxfordjournals.molbev.a003967.
- Tan CH, Wong KY, Tan NH, Ng TS, Tan KY. 2019. Distinctive Distribution of Secretory Phospholipases A2 in the Venoms of Afro-Asian Cobras (Subgenus: *Naja*, *Afronaja*, *Boulengerina* and *Uraeus*). *Toxins*. 11(2).
- Tohamy AA, Mohamed AF, Abdel Moneim AE, Diab MS. 2014. Biological Effects of *Naja haje* Crude Venom on the Hepatic and Renal Tissues of Mice. *Journal of King Saud University – Science*. 26(3): 205–12.
- Topalı, Özbek Bilgin A, Keskin Çimen F, Kurt N, Süleyman Z, Bilgin Y, Özçiçek A, Altuner D. 2018. The Effect of Rutin on Cisplatin-Induced Oxidative Cardiac Damage in Rats. *Anatolian Journal of Cardiology*. 20(3): 136–42.
- Valvona CJ, Fillmore HL, Nunn PB, Pilkington GJ. The Regulation and Function of Lactate Dehydrogenase A. 2016. The Regulation and Function of Lactate Dehydrogenase A: Therapeutic Potential in Brain Tumor. *Brain Pathology (Zurich, Switzerland)*. 26(1): 3–17.
- Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science (New York, N.Y.)*. 324(5930): 1029–33.
- von Zabern I, Przyklenk H, Vogt W. 1982. Chain Structure of Cobra Venom Factor From *Naja Naja* and *Naja haje* Venom. *Scand Immunol*. (4):357-62. doi: 10.1111/j.1365-3083.1982.tb00659.x
- Ward CM, Vinogradov DV, Andrews RK, Berndt MC. 1996. Characterization of Mocarhagin, a Cobra Venom Metalloproteinase from *Naja Mocambique Mocambique*, and Related Proteins from Other Elapidae Venoms. *Toxicon: Official Journal of the International Society on Toxinology*. 34(10): 1203–6.
- WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. Page 317, 318
- Xing JJ, Hou JG, Liu Y, Zhang RB, Jiang S, Ren S, Wang YP, Shen Q, Li W, Li XD, Wang Z. 2019. Supplementation of Saponins from Leaves of *Panax quinquefolius* Mitigates Cisplatin-Evoked Cardiotoxicity via Inhibiting Oxidative Stress-Associated Inflammation and Apoptosis in Mice." *Antioxidants*. 8(9):347.