Egypt. J. Chem. **Vol. 66**, No. 5 pp. 279 – 292 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Anticancer Potentials of Native and Gamma Irradiated Snake Venom against Human Breast and Colon Cancer Cell Lines: In Vitro Study

Maysa M. Mohamed [a,*], Sanaa O. Abdullah[b], Aly F. Mohamed[c], Serag Eldin I. Elbehair[d,e], Heba M. Karam[f], and Mohamed A. El-Desouky[g,*]

[a] *Vairal Media Lab, Egyptian Organization for Biological Products & Vaccines (VACSERA), Giza, 12654, Egypt*
[b] *Professor of O rganic Chemistry, Faculty of Science, Cairo University, City, Giza, Egypt*
[c] *The International Center for Training & Advanced Researches, ICTAR, Cairo, Egypt*
[d] *Biology Department, Faculty of Science, King Khalid University, KSA*
[e] *Cell Culture Lab, Egyptian Organization for Biological Products & Vaccines (VACSERA), Giza, Egypt*
[f] *National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.*
[g] Professor of Biochemistry, Faculty of Science, Cairo University, City, Giza, 12613, Egypt

Abstract

Snake venom includes various elements and therapeutically relevant nontoxic components, and a large number of pharmacologically beneficial compounds have been isolated and described. The purpose of this study was to compare the effects of native and gamma-irradiated *Cerastes cerastes* venom on breast & colon cancer cell lines. The anti-proliferative impact of the native and gamma-irradiated venoms determined using 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. Quantitative real time PCR used to evaluate the expression of apoptosis-related genes. Biochemical analysis Malondialdehyde (MDA) measured by the thiobarbituric acid assay, reduced Glutathione (GSH) was measured photometrically & ROS was assessed by 2, 7- dichlorofluorescein diacetate. Analytical SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Ultraviolet absorption Spectrum 200-900 nm. $IC_{50}$of gamma-irradiated venom was significantly reduced than native venom recorded half maximal inhibitory concentration (IC_{50}) values of (19.93 ± 0.867 & 2.131 ± 0.095) µg/ml and (8.921 ± 0.515 & 0.9454 ± 0.055) µg/ml against human breast cancer (MCF-7) and human colon cancer (HCT-116) cell lines, respectively. Both venoms significantly induced the down-regulated Bcl-2 gene and up-regulated P53 and Bax genes. Both venoms significantly enhanced the antioxidant enzymes' activity levels of H_2O_2, while insignificantly reduced GSH enzyme activity. MDA was significantly elevated post treatment of native venom compared to irradiated venom and was a negative control for both cell lines. Gamma radiation can create changes in the components of snake venom, allowing it to be detoxified; gamma radiation has a significant effect on decreasing venom toxicity while having no influence on immunogenicity in the examined cell lines.

Keywords: : Native; gamma-irradiated; MCF-7; HCT-116; MTT; anti-proliferative; quantitative real time PCR.

1. Introduction

Animal venoms are complex chemical combinations comprising pharmacologically active components with particular biological functions [1], as well as certain non-protein structures such as carbohydrates, lipids, metal inorganic ions including Na+, Ca++, K+, Mg++, Zn++, Fe++, Co++, Mn++, and Ni++, and other unexplained sub-[2]. Venoms are both immunogenic and toxic, making anti-venom production difficult. As a result, using detoxified venoms can be advantageous in a variety of ways [3], but it is important to verify that the detoxified venoms maintaining immunogenicity.

Gamma radiation has been shown to be effective in reducing venom toxicity while maintain immunogenicity [4]. Because macromolecule structure and biological function are so inextricably connected, it appeared that some change was the most plausible explanation for the radiation impacts. Irradiation does not generally induce protein function loss by breaking peptide bonds or altering the peptide chain's basic skeletal structure. It can be produced by

*Corresponding author e-mail: maysa.mahammed@gmail.com .; (Maysa M. Mohamed)
meldesouky@sci.cu.edu.eg; (Mohamed A. El-Desouky).
Receive Date: 25 July 2022, Revise Date: 09 August 2022, Accept Date: 20 August 2022.
DOI 10.21608/ejchem.2022.152363.6599

a rupture in H2 or S-S bonds, which can disrupt internal side chain linkages or expose amino-acid groups, resulting in a biological function change [5]. Purified and defined chemicals generated from snake venom have recently demonstrated great promise as cancer-targeting treatment [6].

Several techniques of venom detoxification have been researched, and one such study is described here [7] suggested that low-dose γ radiation might be used as a supplemental therapy in combination with anti-venoms [8]. There are several causes of sickness and mortality, but cancer remains at the top of the list. Therefore, the innovation of modern therapies or drugs is a high priority [9]. So, the aim of this work was to compare the detoxification effect of gamma ray to snake venom and related toxicity, anti-proliferative potential, and related apoptotic and anti-apoptotic gene profiles post MCF and HCT-116 cell treatment, as well as related cell cycle and antioxidant profiles, to that induced by using native venom and untreated cells.

2. Material and Methods

a. Snake Venom

Lyophilized *Cerastes cerastes* venom was acquired by the laboratory animal department of VACSERA, Egypt. To remove the venom, the milking method was employed. The venom was vacuum-sealed and kept at +4 °C until it was needed [8].

b. Irradiation Venom process

In a saline solution, *Cerastes cerastes* venom (1 mg/ml) was dissolved. Samples were subjected to a radiation dose level of 2 KGy using a cobalt-60 Indian gamma cell [GE 4000A], at the National Center for Radiation Research and Technology (NCRRT). The radiation dose rate was 1.26 Gy/sec at the time of the experiment. This dose was carefully chosen as it reduces venom toxicity while maintaining immunogenicity [10].

c. Cancer cell lines

The MCF-7 and HCT-116 cell lines were provided by the cell culture unit of VACSERA, Egypt. The cell lines for this investigation were obtained from the American Type Culture Collection (ATCC). The cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at a final ambient pressure of 95% air and 5% CO2 [11]. The medium, fetal calf serum (FBS), and additional components were given by Sigma-Aldrich-USA in Egypt.

d. In vitro cytotoxicity

The MCF-7 and HCT-116 cell lines were processed according to the manufacturer's protocol. Cells were seeded in 96 well tissue culture plates (Nunc, USA) at 1x105/ml and incubated at 37 oC for 24 hours. The culture medium was decanted and a fresh se-rum-free medium was dispensed at 0.1 mL/well. Native and irradiated venom were added to the first column of the plates and were two-fold diluted (0.01 to 100 µg/ml). Negative control was included. Plates were incubated over night at 37° C in a 5% CO2 incubator (Jouan, France). 24 hours post cell treatment, treatment medium was de-canted and plates were washed using phosphate buffer saline (PBS) by ADWIA, Egypt. The MTT stain solution (0.5 mg/ml) was used as 25µL/ well (Sigma-Aldrich, USA) was added to each well and the plates were incubated at 37oC for 4 hours. To dissolve the formed formazan crystals, 25µL of dimethyl sulfoxide (DMSO) (BDH, England) was dispensed. The plates were shaken on a plate shaker (Staurt, England) at room temperature for 20 min. The plates were read using an ELISA microplate reader at 570 nm (Dynatec medical products, England). The IC_{50} value was calculated using Sigma Plot 11.0 software [12].

e. Cell cycle analysis

Cell cycle analysis is carried out using the approach described by El Sharkawi et al., [12]. Each cell line (MCF-7, HCT-116) was planted in 96-well plates at a density of 6 x 105 cells per well for 24 hours. The cells were subjected to 19.93 and 2.13 µg/ml for MCF-7 and 8.92 0.945 µg/ml for HCT-116 cell line of native and irradiated venoms respectively for 24 hours. 2.5 x 105 cells were collected and washed two times in ice-cold PBS before being resuspended in 2 x 102 ml of cold PBS. For rapid cell fixation and dispersion, the cell suspensions were added dropwise to 1 ml of cold 70% ethanol and incubated on ice for 45 minutes. After centrifugation, the cells were resuspended in 1 ml of propidium iodide (PI) master mix (PBS containing 100 mg/ml RNase A and 40 mg/ml PI) and incubated for 30 minutes in the dark at 37 ° C. To follow the various phases of the cell cycle, a FACSCalibur flow cytometer was used (BD Biosciences, San Jose, CA). At 488 nm, an argon laser was used to excite cells, and the fluorescence from 10,000 cells was captured using a 580 nm band-pass

filter (FL2-H). The cell cycle profiles were analyzed by Cell Quest version 3.2 and Win MDI version 2.8.

f. Quantitative real-time PCR (qRT-PCR) analysis for genes expression

The IC_{50} values of the tested native and irradiated venoms were compared to those of the cell control. For the experiment, MCF-7 and HCT-116 were treated for 24 hours with the IC_{50} of test venoms to evaluate the apoptotic effects of the venoms on tumour cell lines. The treated cells' RNA was extracted. Control cells that have not been treated, as well as treated cells that have not been handled. Apoptosis-related genes are active. Total RNA was extracted from control, native, and irradiated venoms using the RNeasy Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The concentration of extracted RNA was determined using a Beckman dual spectrophotometer (Beckman Instruments, Ramsey, MN, USA). The expression levels of apoptosis-related genes; P53 [F: 5'-TCA GAT CCT AGC GTC GAG CCC-3' & R: 5'-GGG TGT GGA ATC AAC CCA CAG-3'], BCL2 [F: 5'-GTG AAC TGG GGG AGG ATT GT-3'& R: 5'-GGA GAA ATC AAA CAG AGG CC-3'] and Bax [F: 5′- R: 5′-CCC AGT TGA AGT TGC CGT CA-3′ & R: [R:ATG GAC GGG TCC GGG GAG CA-3′] and housekeeping gene; ACTB [F 5' AGCGAGCATCCCCCAAAGTT- 3'& R: 5' GGGCACGAAGGCTCATCATT-3] were determined using real-time PCR. A total of 10 ng RNA was isolated from each sample and used for cDNA synthesis with a high capacity cDNA reverse transcriptase kit (Applied Biosystems-Thermo Fischer Scientific, USA). Following that, the cDNA was amplified as follows using the Sybr Green I PCR master kit (Thermo Fisher Scientific Inc., Lithuania) and the Step One equipment (Applied Biosystems, Thermo Fisher Scientific): 10 minutes at 95°C for enzyme activation, followed by 40 amplification cycles of 15 seconds at 95°C, 20 seconds at 55°C, and 30 seconds at 72°C. The DCT technique was used to normalize differences in the expression of each target gene relative to the mean critical threshold (CT) values of b-actin, the housekeeping gene [13].

g. Biochemical analysis

Cells treated with MCF-7 and HCT-116 IC_{50}were collected, washed twice with cold PBS, and pelleted in a chilled centrifuge at 1500 RPM for 15 minutes (Jouan-Ki 22, France). The cell lysate was made by resuspending the pelleted cell pellet in 200 L of cold PBS, sonicating in an ice path, and cold centrifuging at 4,000 xg for 15 minutes at +4 °C. The test was performed on the supernatant. Malondialdehyde (MDA), reactive oxygen species (ROS), and glutathione reductase (GR) activity levels were measured using Bio-diagnostic (Cairo, Egypt) prepared kits and the Milton Roy spectronic 21D UV-Visible. MDA and antioxidant enzyme activity per mg of protein were measured [11].

h. Analytical SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A slab-thick gel containing 10% acrylamide was produced using the (staining and destaining) de-staining solution, 10% separating gel with SDS, and 4% stacking gel with SDS. After the lower gel's polymerization was done, the solutions for the top, large-pore gel were combined in proportion. The upper gel solution was poured over the separating gel in the presence of a plastic comb to produce 10 wells after the water layer on top of the lower gel was removed. Each well has a capacity of 100–200 µl. The native venom samples were boiled in sample buffer. The tagged gel was removed from the plates, stained with Coomassie Brilliant Blue R-250, and then a destaining solution was applied. According to Bocian et al., [14] the covalently attached dye alters the appearance of the M.W-based protein and has a propensity to produce bigger bands.

i. UV-Absorption Spectrum

Native and irradiated snake venom in a concentration of 1mg/ml in saline solution (0.15 M NaCl) were recorded on an Ultrospec III spectrophotometer, using a range of UV wavelengths from 200 to 900 nm. Absorption spectra were automatically registered and absorbance was obtained using saline solution (0.15 M NaCl) as a blank [15].

j. Estimation of Total Protein

A Bradford Assay: Using BSA solution from the 2-D Quant Kit, a six-point calibration curve in the range of 0.2 to 2 µg/µL was conducted. Both venoms were diluted 200 times for examination, 1.5 mL of Bradford reagent was added to all samples, and absorbance at 595 nm was recorded after 20 minutes. The standard curve was replicated, and the samples were produced three times. After 20 minutes, absorbance at 595 nm

was measured. The standard curve was replicated, and samples were made in triplicate [16].

k.Statistical analysis

All 3 experiments were conducted 3 times independently. The IC_{50} values were calculated using Graph Pad Prism7 (Graph Pad software, La Jolla, CA, USA). Using an unpaired Student t-test, the P values were calculated by comparing the control cells to each treated cell. The differences were deemed statistically significant at ($P < 0.05$).

3. Results

a. Cytotoxicity

Evaluation of the cytotoxic activity of *Cerastes cerastes* native and irradiated venoms on MCF-7 and HCT-116 cancer cells using the MTT assay revealed that viability was concentration and cell type dependent, as viability increased as long as the venom concentration decreased, figure (1), and the IC_{50} of irradiated snake venom was significantly reduced than in the case of native venom, recording IC_{50} values of (19.93 ± 0.867 & 2.131 ± 0.095) µg/ml and (8.921 ± 0.515 & 0.9454 ± 0.055) µg/ml against MCF-7 and HCT-116, respectively, Table (1).

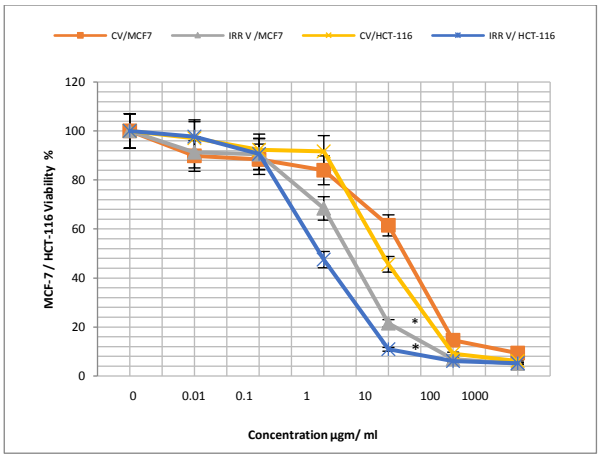


Fig. 1. A: Evaluation of MCF-7 & HCT-116 cancer cell lines viability percentage under the effect of native (CV) and irradiated snake venom (IRR V) relative to concentration.

* IRR V for both cell lines is more significant.

Table 1
Evaluation of IC_{50} (µg/ml) of native & irradiated snake venom relative to MCF-7 & HCT-116 cancer cell line.

Cancer cell line	IC_{50} µg/ml MCF-7±SD	IC_{50} µg/ml HCT-116 ±SD
Native venom	19.93±0.867	8.921±0.515
Irradiated venom	2.131±0.095	0.9454±0.055

Results were expressed as mean ± SD for three different independent replicates using sigma Plot.

b. Cell Cycle

The effect of native and irradiated venom on MCF-7 and HCT-116 cell cycle profiles indicated that the cellular DNA distribution 24 hours post treatment with the IC_{50} values indicated that there was an insignificant increased ($P>0.05$) cellular DNA distribution detected during the G0-G1 (72.19% & 72.66%) , S (26.52% & 25.22%) for MCF-7 cell line, while, HCT-116 cell line, a significant decreased ($P<0.05$) cellular DNA detected during the G0-G1 (29.66% & 47.25%), S (17.98 & 16.33) phases in the case of treatment with both native and irradiated venom compared with its values in the case of untreated cell control. In the mean times, there was a significant ($P<0.05$) cellular DNA distribution occurred in the G2-M phase post cellular treatment with both native and irradiated venoms (52.36% and 36.22%) and a significant arrest of DNA at the G2/M phase in the case of native venom treatment than in the case of cell treatment HCT-116 with irradiated venom as shown in figure, figure (2 & 3).

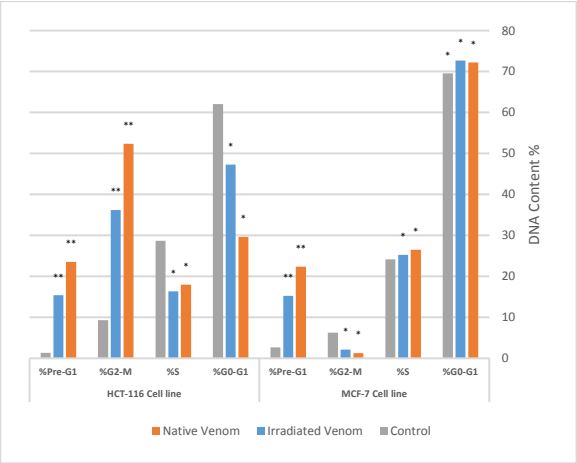


Fig. 2: Cell cycle analysis evaluation of HCT-116 & MCF-7 cells treated with native and irradiated venom using flow cytometry.
*Moderately significant than control. ** Significantly elevated than control.

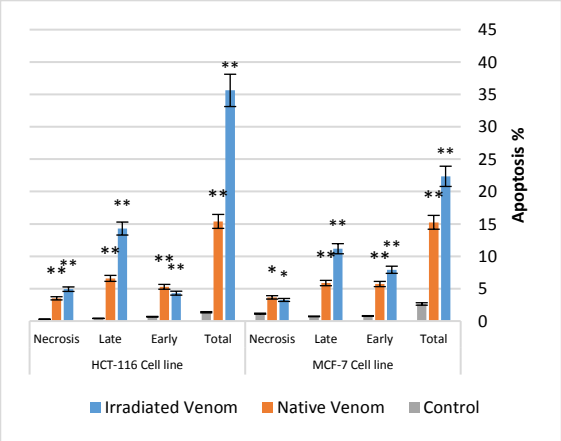
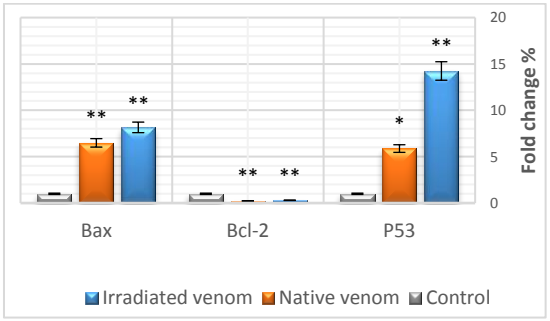


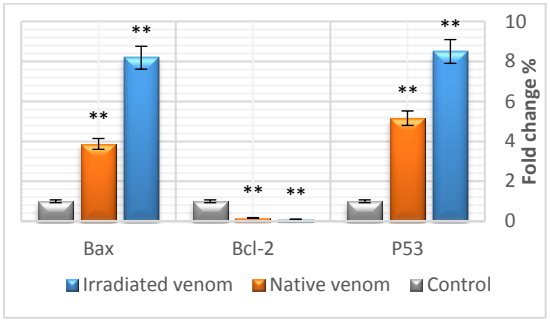
Fig. 3: Apoptotic activity evaluation of HCT-116 & MCF-7 post cell treatment with native and irradiated venom.
*Moderately significant than control. ** Significantly elevated than control.

c. Gene expression

The anticancer potential based on the up regulation and downregulation of pro-apoptotic and anti-apoptotic genes showed that there was a significantly ($P < 0.05$) upregulated P53 gene as compared to the value in the untreated cell control. The overexpression of the P53 gene was substantially greater in irradiated venom-treated cells than in native venom-treated cells. Furthermore, the expression of the P53 gene was considerably higher in MCF-7 than in HCT-116 ($P <0.05$). While its profile in MCF-7 and HCT-116 was insignificantly changed in the case of cell treatment with native venom ($P > 0.05$), in the meantime, there was no significant difference ($P > 0.05$) in the rate of Bax upregulation in MCF-7 and HCT-116 irradiated venom treated cells. Oppositely, Bax was significantly up-regulated ($P <0.05$) in the case of MCF-7 treatment with native venom but not in the case of HCT-116 cell treatment. Finally, the anti-apoptotic gene, Bcl-2, showed a significant ($P <0.05$) downregulation in the case of HCT-116 treated with irradiated and native venom than in the case of MCF-7 cells, figure 4 (A - B).



(A)



(B)

Fig. 4(A-B)
(A): Evaluation of fold change percentage for apoptosis related genes (P53, Bcl-2, and Bax) in MCF-7 cell line treated with irradiated and native *Cerastes cerastes* snake venom against control. (B): Evaluation of fold change percentage for apoptosis related genes (P53, Bcl-2, and Bax) in HCT-116 cell line treated with irradiated and native *Cerastes cerastes* snake venom against control.
*Moderately significant than control. ** Significantly elevated than control.

d. Biochemical Analysis

Lipid peroxidation was monitored via evaluation of the MDA, H_2O_2 and GSH levels as biomarkers of oxidative stress and cellular damage that is evoked by stress. At 24 hours, the venom IC_{50} treatment of MCF-7 and HCT-116 cell lines induced a significant increase in MDA in the HCT-116 cell lysate compared to native venom in the case of irradiated and MCF-7 and negative control cells ($P \leq 0.05$). Also, GSH was insignificantly reduced in both cell lines compared

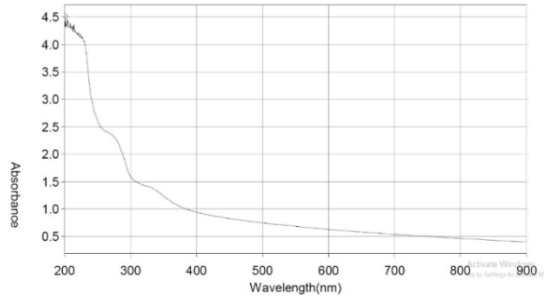
with the negative control (P≥0.05). Finally, H_2O_2 significantly elevated in MCF-7 than in HCT-116 (P≤0.05) and both were significantly elevated compared to cell control (P≤0.05) Table 2.

Table 2
Effect of IC_{50} concentration of test venoms on the antioxidant profile compared to untreated cell control.
P value (< 0.05) indicate significant difference compared to negative control.

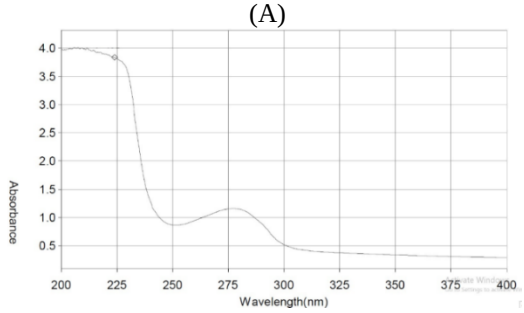
Antioxidant	H_2O_2 (mg/ml)± SD		GSH (mg/ml)± SD		MDA (µM/ml)± SD	
Treatment	MCF-7	HCT-116	MCF-7	HCT-116	MCF-7	HCT-116
Native venom	1.868± 0.0059	0.7006± 0.0077	0.5194± 0.0030	0.4587± 0.0027	1.906± 0.0188	3.33± 0.0275
IRRV	1.602± 0.0207	0.469± 0.0087	0.5982± 0.0061	0.6148± 0.0039	1.754± 0.0179	1.798± 0.0198
Control	0.5477± 0.0310	0.3436± 0.0350	0.9130± 0.0114	0.9587± 0.0318	0.684± 0.0325	1.069± 0.0145

e. UV- Spectrophotometric Analysis

There are different peaks in native and irradiated venoms. Spectrophotometer Thermo Scientific Helios Gamma was used in the analysis at "CURP", Faculty of Agriculture, Cairo University, figures 5 (A & B).



(A)



(B)

Fig. 5 (A-B)
(A): UV Spectrum of *Cerastes cerastes* native venom begins at 200 nm and ends at 900 nm with absorbance begins at 4.523 nm and ends at 0.401 nm.
(B): UV Spectrum of *Cerastes cerastes* irradiated venom begins at 200 nm and ends at 400 nm with absorbance begin 3.979 nm and ends at 0.291 nm.

f. Total protein in native Venom and irradiated venom

The protein concentration of native and irradiated venoms was 13.00 mg/ml and 11.3 mg/ml, respectively. In figure (6), there was an insignificant difference in total protein concentration between native and irradiated venom.

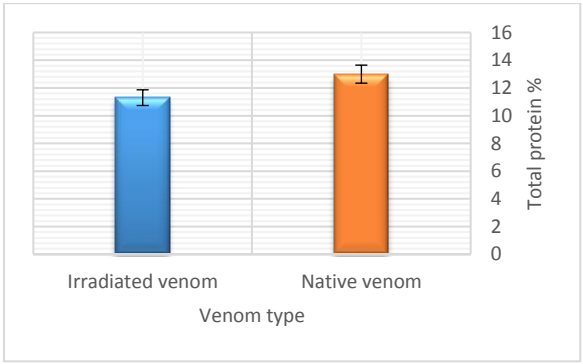


Fig. 6: Total Proteins of native and irradiated *Cerastes cerastes* snake venom in mg/ml.

g. SDS-PAGE profiles of venoms

SDS-polyacrylamide gel electrophoresis reported that the venom components were altered after irradiation, some protein bands gradually disappeared, and the intensity of bands decreased. Electrophoretic separation of venom was performed under reducing conditions and non-reducing conditions.

In the present result native venom reported different band 30,60, 72, 95, 130 KD and irradiated venom reported that 26,72 &130 KD but in and non-reducing

conditions native venom 30, 55 & 72 KD irradiated venom 30, 60 KD that represented in figure (7).

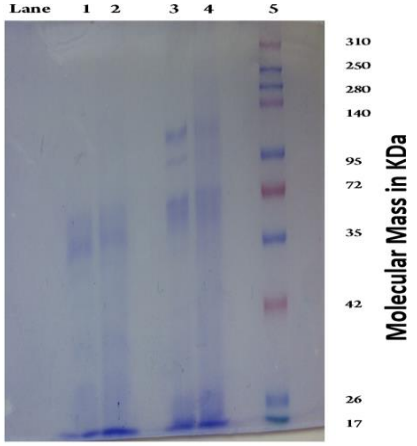


Fig. 7: SDS-PAGE profiles of venoms. Electrophoretic separation of venom was performed under reducing and non-reducing conditions.
Lane 1: Native venom under reducing conditions. Lane 2: Irradiated venom under reducing conditions. Lane 3: Native venom under non-reducing conditions. Lane 4: Irradiated venom under non- reducing conditions. Lane 5: Molecular mass markers KDa.

4. Discussion

Cancer is one of the major public health problems around the world, and finding new cancer treatments is a major research focus worldwide. Several studies have found that snake venoms possess therapeutic agents that can be used as anticancer agents [17]. They account for approximately 1.19 percent, 2.17 percent, and 4.77 per-cent of all malignancies in Lower, Middle, and Upper Egypt, respectively [18]. Snake venoms are complex mixtures containing different peptides, proteins, enzymes, carbohydrates, and other bioactive molecules, which are secreted by the snake during predation or defending against threats [9]. Snake venoms can be considered as mini-drug libraries in which each drug is pharmacologically active. However, less than 0.01% of these toxins have been identified and characterized. For instance, CaptoprilR (Enalapril), IntegrilinR (Eptifibatide) and AggrastatR (Tirofiban) are drugs based on snake venoms that have been approved by the FDA. In addition to these approved drugs, many other snake venom components are now involved in preclinical or clinical trials for a variety of therapeutic applications. These examples show that snake venom can be a valuable source of new principle components in drug discovery [19].

Snake venoms have the potential to open up new avenues for medication development and cancer research [20]. Snake venoms are more complex than the venoms of other species such as spiders, scorpions, and cone snails [21]. The pharmacological effects of these animal venoms are mostly due to disulfide bridged peptides, however snake venoms contain a more diverse variety of larger proteins and peptides, resulting in a wider range of pharmacological and toxicological effects [22]. *Cerastes vipera* is one of Egypt's most poisonous snakes. The Sahara Sand Viper (*Cerastes vipera*) is a venomous viper indigenous to North African deserts and the Sinai Peninsula [23].

Due to the fast development of resistance to chemotherapeutic treatments, the hunt for innovative medications remains a top aim for cancer therapy. Furthermore, the high toxicity of some cancer chemotherapeutic treatments, as well as their unfavorable side effects, raises the demand for innovative anti-tumor therapies that are active against untreatable tumors, have fewer side effects, and/or have superior therapeutic efficacy [24].

Cerastes cerastes venom is rich in active disintegrin fractions and exerts a higher cytotoxic effect on all cell lines. It is a poisonous and widely distributed snake in Africa and inhabits the sandy deserts of Egypt. It has therapeutic potential in the treatment of cancer, especially through exerting cytotoxic, antiangiogenic, and apoptotic effects [25]. Some venom peptides have a high cytotoxic effect and target cancer cells over healthy cells [26]. Snake venoms show the highest cytotoxic potential, suggesting a promising use of these venoms or their components as antitumoral agents. The fact that tumour cells have higher cytotoxic and cytostatic activity than normal cells suggests that clinical applications are possible [27].

Gamma irradiation has the potential to alter the molecular structure and affect the biological properties of biomolecules, which is why it has been successfully employed to attenuate animal toxins [28]. Gamma radiation has been shown to be useful for reducing venom toxicity and preserving or, in some circumstances, boosting immunogenicity [29]. Gamma irradiation has been reported as a potent tool for venom detoxification, since the structural changes caused by radiation slow down or stop the enzymatic activities of toxic proteins, while retaining epitope configurations that induce efficient immune responses [8].

In the present study, the viability percentage of cancer cells was used as an indicator of the test venom cell toxicity. It was noticed that toxicity was both concentration and time-dependent, as viability increased as long as the concentration decreased. The related IC_{50}recorded was 19.93 ± 0.867 & 2.131 ± 0.095 μg/ml and 8.921 ± 0.515 & 0.9454 ± 0.055

μg/ml against MCF-7 and HCT-116, respectively, and irradiated venom was significantly more toxic to cell lines than native venom cells, where a cytotoxicity assay (MTT) was carried out to determine the IC_{50} (cytotoxic dose) of the venom samples.

Viability increased as long as concentration decreased, which was in accordance with the report by Mukherjee, et al., [30], that Russell's viper venom has an-ti-proliferative activity against breast cancer.

The recorded present results are in agreement with results of El Sharkawi, et al., [12], who showed cytotoxicity against liver and breast cells of snake venom with (IC_{50} of 5.86 and 13.05 μg /ml). The cytotoxicity of sv LAAOs was much higher than snake venom, with an IC_{50} of 3.65 and 0.48 μg /ml. This finding is in harmony with that of Salama et al., [31], who reported that the anti-cancer activity of an isolated LAAO from the closely related *Cerastes vipera* venom against MCF-7 cells was reported with an IC_{50} value of 2.8 μg/ml.

Furthermore, the cytotoxic, anti-proliferative and apoptotic activity of an LAAO from the Malaysian *C. purpureomaculatus* was screened against colon cancer cells (SW480 and SW620). They reported an IC_{50} value of 13 μg/mL against colon cancer cell lines and demonstrated morphological changes as well as apoptosis [32], which is consistent with our findings.

According to Ozverel et al., [26], cytotoxicity studies of SHSY5Y cells with purified venom fractions revealed heterodimeric disintegrins from *C. cerastes* venom, which exerted a high cytotoxic activity with IC_{50} values from 0.11 to 0.58 μg/ml and a disintegrin-like effect on SHSY5Y morphology was observed due to cell detachment. Different studies showed that the cytotoxic, anti-proliferative, and apoptotic effects of different snake venoms against different cancer cell lines were revealed in the potential of venoms to act against an epithelial cancer cell line. The effect of venom on the viability of PC3 cells was evaluated and was concentration dependent [33].

Our results regarding the increased cytotoxic potential of irradiated and native venom, elevated toxicity, and cell type dependency were agreed with [34]. The cytotoxicity of snake venom to cancer cells may be attributed to the venom proteolytic enzymes that were in accordance with Tavares, et al., [35], despite the cell type difference, recording that amino acid oxidase from *C. rhodostoma* snake venom was cytotoxic to HEL92.1.7 and SET-2 cells IC_{50}% of 0.15 mg/ml and induced apoptotic activity in a concentration-dependent manner.

The relationship between native and irradiated venom and the related elevation of anti-oxidant levels enhanced the pro/anti-apoptotic gene expression and cancer cell DNA accumulation, indicating the cell cycle arrest or cellular DNA accumulation was induced at the G2/M phase. Few studies have been conducted to assess the effects of LAAO on cell cycle progression. They evaluated the cycle modulation and the induction of apoptosis in cells treated with LAAO, reporting that this toxin induced a delay in the G0/G1 phase and that this delay may prevent the initiation of DNA synthesis and, consequently, the replication of tumour cells, which could represent another possible mechanism by which LAAO displays its antitumor effects [36].

The effect of native and irradiated venom may be due to the presence of LAAO, which showed an accumulation of tumour cells in the sub-G1 phase of the cell cycle. Our findings agreed, as it was noticed that there was a significant accumulation of cellular DNA induced during the G2/ M phase in a significant compared with the arrest induced in untreated cell control [37]. The cross-like between Bcl-2 and P53 genes is a significant determinant of drug-induced apoptosis [38].

The present results showed that a 24-hour treatment of MCF-7 and HCT-116 cells with both venoms, native and irradiated, caused a significant down-regulation of Bcl-2 and an up-regulation of P53 compared to control cells. These results agreed with studies that have reported the down-regulating effect of some snake and scorpion venoms on Bcl-2 gene expression [39].

Our results agreed with Pang et al., [41], who reported that the decreases in Bcl-2 genes in HCT-8 cancer cells after treatment with *Agkistrodon acutus* snake venom, The down-regulating effect caused by the venoms on Bcl-2 is noteworthy since Bcl-2 is a highly conserved member of the Bcl-2 family, and it constitutes an important regulator of apoptosis. By blocking cytochrome c release from mitochondria, Bcl-2 protects cells from apoptosis by inhibiting the activation of the caspase 3 dependent pathway. This is because Bcl-2 is not only a mediator of apoptosis, but is also involved in programmed necrosis. The Bcl-2 family proteins, including Bcl-2 and Bax, contribute to the regulation of apoptosis. In particular, anti-apoptotic members of the Bcl-2 family, such as Bcl-2, act to prevent or delay cell death, whereas the proapoptotic Bax promotes apoptosis [41].

The levels of Bax were markedly elevated in association with both venoms in comparison to the control group, which indicates an active process of apoptosis via activation of Bax through the mitochondrial pathway. In agreement with our results, induction of the mitochondrial apoptosis pathway and

over-expression of caspase-3 in comparison to untreated groups were revealed with Naja haje venom, [42] *Cerastese cerastese* venom.

Concerning the anticancer potential of snake venoms and despite the use of different cancer cell line (PC3) and venom other studies reported that V. lebetina snake venom inhibited the growth of human prostate cancer cells by induction of apoptosis through inhibition of nuclear factor KB (NF-KB), and increased the expression of pro-apoptotic genes (p53, Bax, caspase-3, and caspase-9), and down-regulated anti-apoptotic (Bcl-2) gene [43].

According to the results, the revealed biological activity of irradiated and native venom enhances the intrinsic apoptotic pathway as P53 as a protein can play a critical role in the intrinsic apoptosis pathway activation and the induction protein was found to be stuck in the presence of Bcl-2. That is in agreement with other studies that re-ported the cross-like between Bcl-2, P53, and Bax genes is a significant determinant of drug-induced apoptosis [42].

Also, it was proved that snake venom showed up-regulation of pro-apoptotic genes and down-regulation of anti-apoptotic genes [44], indicating that snake venom toxin also increased the expression of pro-apoptotic protein Bax but down-regulated anti-apoptotic protein Bcl-2. The pro-apoptotic and anti-apoptotic gene levels were elevated under the effect of native and irradiated venom. This was explained by the regulation of the apoptotic mitochondrial pathway, which occurs through members of the Bcl-2 family of proteins that can either be pro-apoptotic or anti-apoptotic. According to Adams and Cory [45], the tumour suppressor protein P53 has a critical role in the regulation of the Bcl-2 family of proteins. Different studies have reported that exposure of cancer cells to toxins/venoms increases the levels of oxidative markers [33]. A significant increase in MDA in HCT-116 and MCF-7 cell lines in native venom com-pared to irradiated venom and negative control cells ($P \leq 0.05$). This agrees with reports that showed an elevation of lipid peroxidation levels after treatment with various types of venoms [46]. GSH was insignificantly reduced in both cell lines compared with the negative control ($P \geq 0.05$). Dkhil, et al., [47] found that *Naja h.* venom induced renal toxicity to rat kidney tissue through decreasing GSH content by 41.02% and through the reduction of GR, GST, and GPx gene expression. In another study, reduced GSH in the liver tissue of envenomed rats with LD_{50}% *Naja h.* venom was found to be due to a decrease in GR expression.

Finally, H_2O_2 was significantly elevated in MCF-7 than in HCT-116 and both were significantly elevated compared to cell control.

The apoptotic effect of irradiated and native venom of *Vipera lebetinaturanica* can induce apoptosis in many cancer cell lines, including human neuroblastoma cells. In-creased reactive oxygen species (ROS) were observed in SK-N-MC and SK-N-SH cells, and mitochondria membrane potential (MMP) was also disrupted in V. lebetina-treated neuroblastoma cells [48].

Regarding the anticancer activity of venoms based on the elevated or non-elevated levels of antioxidants, our data was in agreement with Al-Asmari, et al., [49], who found that different snake venoms could cause apoptosis in colon and breast cancer cell lines through increasing ROS. However, in contrast to our results, other studies showed decreases in some antioxidant enzymes in the case of treatment with venoms.

Antioxidant inhibitors and/or reactive oxygen species (ROS) generating com-pounds induce apoptosis in cancer cells. In accordance with Franco and Cidlowski, [50], our results show that a 24 hour treatment of MCF-7 and HCT-116 cells with the IC_{50} of these venoms caused a significant increase in the activity of the antioxidant enzymes in the cell lines compared to the control cells. This agrees with reports that showed an increase in lipid peroxidation levels and antioxidant enzymes when using venom as a treatment.

The present study provides evidence supporting the theory that GSH, H2O2 and MDA play an important role in the genotoxicity of venom treatment cells.

In the present study, UV absorption spectrum and protein concentration analysis showed that irradiated snake venom spectrum presented differences when compared to native snake venom, suggesting that some structural alterations have occurred.

The UV-visible spectrum of irradiated and native venom showed a typical spectrum of the protein, which has an absorbance maximum of around 200–900 nm. Sadawe, et al., [15] reported that the cobra protein main chain absorbs light in the region of 240–340 nm. The aromatic side chains of cobra venom contain tyrosine, tryptophan, and phenylalanine, which are responsible for the absorbance in this region. It was also reported that UV-vis absorption profiles for monomeric proteins rich in charged amino acids spanning 250–800 nm have opened a new label-free optical spectral window for probing biomolecular structure and interactions [51].

In the current study, the protein concentrations of native and irradiated venoms were 13.00 mg/ml and 11.3 mg/ml, respectively, with an insignificant difference in total protein concentration between the two. The total number of protein bands was found to be lower in Viperidae snakes and it was observed that low molecular weight proteins are metalloproteins and

are responsible for major local symptoms in snake-bite, causing hemorrhage, inflammation, edema, hypotension, hypovolemia, and necrosis [52]. Cho and Song, [53], reported that chemical changes in the proteins that are caused by gamma-irradiation are fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water. Moreover, Minton and Weinstein [54], showed that the SDS-PAG electrophoresis technique for venom obtained 7–10 protein bands of proteroglyph Elapidae and Hydrophidae snake venoms, which is known as complex venom. Ghanghro et al., [52], report that the total number of protein bands was found to be lower in Viperidae snakes, and it was observed that low molecular weight proteins are metalloproteinases and responsible for major local symptoms in snakebite, causing hemorrhage, inflammation, ede-ma, hypotension, hypovolemia, and necrosis. Bennacef-Heffar and Laraba-Djebari [55], reported that electrophoretic analysis of native and irradiated venom with 1 and 2 kGy showed that the native venom revealed 10 bands, each having different molecular masses.

Abdel-Aty et al., [56], reported that the molecular mass of hemorrhagic *Cerastes vipera* metalloprotease was 60 kDa under reducing conditions and was presented as a single band in SDS-PAGE. Chapelier et al., [57], reported that venom components were altered after irradiation, the protein bands disappeared, and a streaking in the gel was observed, and they found that irradiation of α-lact albumin induced the loss of aromatic amino acids and helicity, so fragmentation and aggregation products were obtained.

In the present study, SDS-polyacrylamide gel electrophoresis reported that the venom components were altered after irradiation, some protein bands gradually dis-appeared, and the intensity of bands decreased. Under reducing conditions and non-reducing conditions, electrophoretic separation of venom was performed. El-Yamany, et al., [58], studied that the molecular mass of purified SVMP from Epy venom was 60 kDa under non-reducing and reducing conditions when applied to SDS-PAGE. Abdel-Aty and Wahby [59], who reported that the molecular masses of four isolated metalloproteases from Epy venom were 60, 62, 66, and 67 kDa under non-reducing and reducing conditions, also reported that the molecular mass of hemorrhagic *Cerastes vipera* metalloprotease was 60 kDa under reducing conditions and presented as a single band in SDS PAGE. Dyab, et al., [60], showed a change in the protein bands after irradiating the native *Echis coloratus* at 2, 5, and 10 KGy by subjecting it to SDS-polyacrylamide gel electrophoresis. Samy, et al., [5], reported that the venom components were altered after irradiation, some protein bands gradually disappeared, and the intensity of bands decreased. The SDS-PAGE study indicated that irradiation caused initial fragmentation of the proteins and subsequent aggregation due to cross-linking of the protein molecules. The effect of irradiation on the protein was more significant at lower protein concentrations where gamma-irradiation increased the degradation of the protein molecules [61].

5. Conclusions

Finally, it can be concluded that, The results obtained here show that irradiated venom is reported IC_{50} less than native venom with highly effective result on HCT-116 & MCF-7cancer cell lines, decrease viability of cancer cells, decrease percentage of DNA content in G0-G1 and S, increase percentage of DNA content in G2-M and pro G1 phases in addition to down regulation of Bcl-2 and up regulation of Bax levels and P53 with elevated antioxidant markers those support the DNA damage inducing molecular modulation.

This confirms that gamma radiation can cause changes in the components of snake venom, providing its detoxification; the gamma radiation has important effect on lowering toxicity from venom without effect on immunogenicity as it can change the molecular structure of proteins, after exposure snake's venom.

References

1. Abdel-Aziz, S. A. A; Mohamed, A. F; Zahkouk, S. A. M; Ali R. A. M. Evaluation of Anticancer Activity of Some Venomous Animal Toxins on Human Breast and Colon Cancer Cell Lines and Related Antioxidant Profile. International Journal of Advanced Research 2017, 5 (2): 2036–53. https://doi.org/10.21474/ijar01/3376

2. Burin, S. M.; Menaldo, D. L.; Sampaio, S. V.; Frantz, F. G.; Castro, F. A. An overview of the immune modulating effects of enzymatic toxins from snake venoms. International journal of biological macromolecules, 2018, 109, 664–671. https://doi.org/10.1016/j.ijbiomac.2017.12.101

3. Caproni, P.; Baptista, J.A.; Almeida, T.L.; Passos, L.A.; Nascimento, N. Study of Irradiated Bothropstoxin-1 with 60Co Gamma Rays: Immune System Behavior. J. Ven. Anim. Tox. Incl. Trop. Dis. 2009, 15: 216-225." J. Ven. Anim. Tox., 216–25.

4. Shaban, E. A. Influence of Ionizing Radiation on Cobra (Naja Haje) and *Cerastes Cerastes* Venoms: Toxicological and Immunological Aspects.The Egyptian Journal of Hospital Medicine . 2003,13 (1): 99–111. https://doi.org/10.21608/ejhm.2003.1823
5. Samy, E.M.; Shaaban, E.A.; Kenawy, S.A.; Galal, M.A. ;Salama, W.H. Evaluation of the effect of gamma rays on *Echis Coloratus* snake venom through toxicological , immunological and biological studies. Inter. J. Sci. Res. 2015. Publication, 5: ISSN 2250-3153." J. Sci. Res. Publication 5: 2250–3153.
6. Aarti, C.; Khusro, A. Snake venom as anticancer agent- Current Perspective. Int. J. Pure App. Biosci. 2013, 1 (6): 24-29 .
7. Karam, H.; Mohamed, M. Beneficial effect of low dose gamma irradiation or quercetin on *Cerastes cerastes* snake venom induced toxicity in male rats, Toxin Reviews, 2021, DOI: 10.1080/15569543.2019.1580746.
8. Oussedik-Oumehdi, H.; Laraba-Djebari, F. Irradiated *Cerastes cerastes* Venom as a Novel Tool for Immunotherapy. Immunopharmacology and immunotoxicology. 2008, 30. 37-52. DOI: 10.1080/08923970701812324.
9. Li, L., Huang, J.; Lin, Y. Snake Venoms in Cancer Therapy: Past, Present and Future. Toxins, 2018, 10(9), 346. https://doi.org/10.3390/toxins10090346.
10. Karam, H.; Shaaban, E.; Fahmy, A.; Zaki, H.; Kenawy, S. Improvement of *Naja haje* snake antivenom production using gamma radiation and a biotechnological technique. Toxin Reviews. 2019, 40.1-11. Doi: 10.1080/15569543.2019.1700381.
11. Geretto, M.; Ponassi, M.; Casale, M.; Pulliero, A.; Cafeo, G.; Malagreca, F.; & Profumo, A.; Balza, E.; & Bersimbaev, R.; & Kohnke, F.; & Rosano, C.; & Izzotti, A. A novel calix[4]pyrrole derivative as a potential anticancer agent that forms genotoxic adducts with DNA. Scientific Reports. 2018, 8. DOI:10.1038/s41598-018-29314-9
12. El Sharkawi, F.; Saleh, S.; & Mohamed, A. Potential anti cancer activity of snake venom, bee venom and their components in liver and breast carcinoma. IJPSR, 2015; Vol. 6(8): 3224-3235. Doi:10.13040/IJPSR.0975-8232.6 (8).3224-35.
13. Marino, A.; Battaglini, M.; De Pasquale, D.; Degl'Innocenti, A.; Ciofani, G. Ultrasound-Activated Piezoelectric Nanoparticles Inhibit Proliferation of Breast Cancer Cells. Scientific reports, 2018, 8(1), 6257. https://doi.org/10.1038/s41598-018-24697-1.
14. Bocian, A.; Sławek, S.; Jaromin, M.; Hus, K. K.; Buczkowicz, J.; Łysiak, D.; Petrílla, V.; Petrillova, M.; Legáth, J. Comparison of Methods for Measuring Protein Concentration in Venom Samples. Animals : an open access journal from MDPI, 2020, 10(3), 448. https://doi.org/10.3390/ani10030448.
15. Sadawe, I.A.; Meiqal, N.H.; Bensaber, S.M.; Maamar, M.S.; Hermann, A.; Alshoushan, A.; Gbaj, A.M. Characterization of Libyan Cobra [Naja haje] Venom using Fluorescence and UV-Visible Spectroscopy, Journal of Clinical & Community Medicine, 2020, 129–132. https://doi.org/10.32474/JCCM.2020.02.000127.
16. Marone, M.; Mozzetti, S.; De Ritis, D.; Pierelli, L.; Scambia, G. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. Biological procedures online, 2001, 3, 19–25. https://doi.org/10.1251/bpo20 .
17. Jain, D.; Kumar, S. Snake venom: a potent anticancer agent. Asian Pacific journal of cancer prevention : APJCP, 2012, 13[10], 4855–4860. https://doi.org/10.7314/apjcp.2012.13.10.4855.
18. Blagosklonny, M. Why Therapeutic Response May Not Prolong the Life of a Cancer Patient: Selection for Oncogenic Resistance. Cell cycle (Georgetown, Tex.). 2006, 4. 1693-8. DOI:10.4161/cc.4.12.2259.
19. Ibrahim, A. S.; Khaled, H. M.; Mikhail, N. N.; Baraka, H.; Kamel, H. Cancer incidence in egypt: results of the national population-based cancer registry program. Journal of cancer epidemiology, 2014, 437971. https://doi.org/10.1155/2014/437971.
20. Mohamed Abd El-Aziz, T.; Garcia Soares, A.; Stockand, J. D. Snake Venoms in Drug Discovery: Valuable Therapeutic Tools for Life Saving. Toxins, 2019, 11(10), 564. https://doi.org/10.3390/toxins11100564.
21. Shanbhag, V.K.L. Applications of snake venoms in treatment of cancer. Asian Pac. J. Trop. Biomed. 2015, 5:275–276. doi:10.1016/S2221-1691[15]30344-0.
22. Ferraz, C. R.; Arrahman A.; Xie C.; Casewell, N. R., Lewis, R. J.; Kool, J.; Cardoso, F. C. Multifunctional Toxins in Snake Venoms and Therapeutic Implications: From Pain to Hemorrhage and Necrosis. Frontiers in Ecology and Evolution. DOI=10.3389/fevo.2019.00218.
23. Munawar, A.; Ali, S. A.; Akrem, A.; Betzel, C. Snake Venom Peptides: Tools of Biodiscovery. Toxins, 2018, 10[11], 474. https://doi.org/10.3390/toxins10110474.
24. Saber, S.A; Mohamed, A.F.; El-Fiky, A.A. and Eldaly, H.H. In Vitro Evaluation of Antibacterial Potential of Cerastes Vipera Venom against Gram-Positive and Gram-Negative Bacterial Strains. The

Egyptian Journal of Hospital Medicine, 2019, Vol. 77 [6], Page 5804-5816.

25. Demain, A. L.; Vaishnav, P. Natural products for cancer chemotherapy. Microbial biotechnology, 2011, 4(6), 687–699. https://doi.org/10.1111/j.1751-7915.2010.00221.x.

26. Ozverel, C. S.; Damm, M.; Hempel, B. F.; Göçmen, B.; Sroka, R.; Süssmuth, R. D.; Nalbantsoy, A. Investigating the cytotoxic effects of the venom proteome of two species of the Viperidae family (*Cerastes cerastes* and Cryptelytrops purpureomaculatus) from various habitats. Comparative biochemistry and physiology. Toxicology & pharmacology, 2019, : CBP, 220, 20–30. https://doi.org/10.1016/j.cbpc.2019.02.013.

27. Giribaldi, J.; Smith,J.J.; Schroeder, C.I. Recent developments in animal venom peptide nanotherapeutics with improved selectivity for cancer cells, Biotechnology Advances, Volume 50, 2021, 107769, , https://doi.org/10.1016/j.biotechadv.2021.107769.

28. Calderon, L. A.; Sobrinho, J. C.; Zaqueo, K. D.; de Moura, A. A.; Grabner, A. N.; Mazzi, M. V.; Marcussi, S.; Nomizo, A.; Fernandes, C. F.; Zuliani, J. P.; Carvalho, B. M.; da Silva, S. L.; Stábeli, R. G.; Soares, A. M. Antitumoral activity of snake venom proteins: new trends in cancer therapy. BioMed research international, 2014, 203639. https://doi.org/10.1155/2014/203639.

29. Oliveira, K. C.; Spencer, P. J.; Ferreira, R. S.; Jr; Nascimento, N. New insights into the structural characteristics of irradiated crotamine. The journal of venomous animals and toxins including tropical diseases, 2015, 21, 14. https://doi.org/10.1186/s40409-015-0013-z.

30. Mukherjee, A. K.; Saviola, A. J.; Burns, P. D.; Mackessy, S. P. Apoptosis induction in human breast cancer (MCF-7) cells by a novel venom L-amino acid oxidase (Rusvinoxidase) is independent of its enzymatic activity and is accompanied by caspase-7 activation and reactive oxygen species production. Apoptosis : an international journal on programmed cell death, 2015, 20(10), 1358–1372. https://doi.org/10.1007/s10495-015-1157-6.

31. Salama, W.H.; Ibrahim, N.M.; El Hakim, A.E.; Bassuiny, R.I.; Mohamed, M.M.; Mousa, M.M.; F.M., Ali. L-Amino Acid Oxidase from Cerastes Vipera Snake Venom: Isolation, Characterization and Biological Effects on Bacteria and Tumor Cell Lines. Toxicon: Official Journal of the International Society on Toxinology, 2018, 150, 270–279

32. Zainal Abidin, S. A.; Lee, Y. Q.; Othman, I.; Naidu, R. Malaysian Cobra Venom: A Potential Source of Anti-Cancer Therapeutic Agents. 2019, Toxins, 11(2), 75. https://doi.org/10.3390/toxins11020075.

33. Akef, H.; Kotb, N.; Abo-Elmatty, D.; Salem, S. Anti-proliferative Effects of Androctonus amoreuxi Scorpion and *Cerastes cerastes* Snake Venoms on Human Prostate Cancer Cells. Journal of cancer prevention, 2017, 22(1), 40–46. https://doi.org/10.15430/JCP.2017.22.1.40.

34. Costa, T. R.; Burin, S. M.; Menaldo, D. L.; de Castro, F. A.; & Sampaio, S. V. Snake venom L-amino acid oxidases: an overview on their antitumor effects. The journal of venomous animals and toxins including tropical diseases, 2014, 20, 23. https://doi.org/10.1186/1678-9199-20-23.

35. Tavares, C.; Maciel, T.; Burin, S.; Ambrósio, L.; Ghisla, S.; Sampaio, S.; Castro, F. l-Amino acid oxidase isolated from Calloselasma rhodostoma snake venom induces cytotoxicity and apoptosis in JAK2V617F-positive cell lines. Revista brasileira de hematologia e hemoterapia, 2016, 38(2), 128–134. https://doi.org/10.1016/j.bjhh.2016.03.004

36. De Melo Alves Paiva, R.; de Freitas Figueiredo, R.; Antonucci, G. A.; Paiva, H. H.; de Lourdes Pires Bianchi, M.; Rodrigues, K. C.; Lucarini, R.; Caetano, R. C.; Linhari Rodrigues Pietro, R. C.; Martins, C. H.; de Albuquerque, S.; Sampaio, S. V. Cell cycle arrest evidence, parasiticidal and bactericidal properties induced by L-amino acid oxidase from Bothrops atrox snake venom. Biochimie, 2011, 93(5), 941–947. https://doi.org/10.1016/j.biochi.2011.01.009.

37. Mahmoud, G.; Saber, S.; Elfiky, A.; Mohamed, A. In Vitro Evaluation of Anticancer Potential of Echispyramidum Venom (Viperidae) and Related Genetic and Apoptotic Profile Alterations. The Egyptian Journal of Hospital Medicine, 2019, [4]: 3891-3900. https://doi.org/10.21608/ejhm.2019.41567.

38. Giménez-Bonafé, P.; Tortosa, A.; Pérez-Tomás, R. Overcoming drug resistance by enhancing apoptosis of tumor cells. Current cancer drug targets, 2009, 9(3), 320–340. https://doi.org/10.2174/156800909788166600.

39. Díaz-García, A.; Morier-Díaz, L.; Frión-Herrera, Y.; Rodríguez-Sánchez, H., Caballero-Lorenzo, Y., Mendoza-Llanes, D., Riquenes-Garlobo, Y., & Fraga-Castro, J. A. In vitro anticancer effect of venom from Cuban scorpion Rhopalurus junceus

against a panel of human cancer cell lines. Journal of venom research, 2013, 4, 5–12.
40. Pang, Z.; Wenqing, L.; Ruiren, Z. The Agkistrodon Acutus Venom Componets of X in Vitro Anti-Tumor Effect and Mechanism. Life Science Journal, 2010, 7 [1]: 41–45.
41. Song, J.K.; Jo, M.R.; Park, M.H.; Song, H.S.; An, B.J.; Song, M.J.; Han, S.B.; Hong, J.T. Cell Growth Inhibition and Induction of Apoptosis by Snake Venom Toxin in Ovarian Cancer Cell via Inactivation of Nuclear Factor _B and Signal Transducer and Activator of Transcription 3. Arch. Pharm. Res. 2012, 35, 867–876 .
42. Shebl, R.; Mohamed, A.; Ali, A.; Amin, M. *Cerastes cerastes* and *vipera lebetina* snake venoms apoptotic - stimulating activity to human breast cancer cells and related gene modulation. Journal of Cancer Science and Therapy. 2012, 4. 317-323. Doi: 10.4172/1948-5956.1000161.
43. Son, D. J.; Park, M. H.; Chae, S. J.; Moon, S. O.; Lee, J. W.; Song, H. S.; Moon, D. C.; Kang, S. S.; Kwon, Y. E.; & Hong, J. T. (2007). Inhibitory effect of snake venom toxin from Vipera lebetina turanica on hormone-refractory human prostate cancer cell growth: induction of apoptosis through inactivation of nuclear factor kappaB. Molecular cancer therapeutics, 6(2), 675–683. https://doi.org/10.1158/1535-7163.MCT-06-0328.
44. Park, M. H.; Jo, M.; Won, D.; Song, H. S.; Han, S. B.; Song, M. J.; Hong, J. T. Snake venom toxin from Vipera lebetina turanica induces apoptosis of colon cancer cells via upregulation of ROS- and JNK-mediated death receptor expression. BMC cancer, 2012, 12, 228. https://doi.org/10.1186/1471-2407-12-228.
45. Adams, J. M.; Cory, S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene, 2007, 26(9), 1324–1337. https://doi.org/10.1038/sj.onc.1210220.
46. Santhosh, M. S.; Sundaram, M. S.; Sunitha, K.; Jnaneshwari, S.; Devaraja, S.; Kemparaju, K.; Girish, K. S. Propensity of crocin to offset Vipera russelli venom induced oxidative stress mediated neutrophil apoptosis: a biochemical insight. Cytotechnology, 2016, 68(1), 73–85. https://doi.org/10.1007/s10616-014-9752-x.
47. Dkhil, M. A.; Al-Quraishy, S.; Farrag, A. R. H.; Aref, A. M.; Othman, M. S. and Moneim, A. E. A. Oxidative Stress and Apoptosis Are Markers in Renal Toxicity Following Egyptian Cobra [Naja Haje] Envenomation. Pak. J. Zool., 2014, 46[6]: 1719-1730.
48. Park, M. H.; Son, D. J.; Kwak, D. H.; Song, H. S.; Oh, K. W.; Yoo, H. S.; Lee, Y. M.; Song, M. J.; Hong, J. T. Snake venom toxin inhibits cell growth through induction of apoptosis in neuroblastoma cells. Archives of pharmacal research, 2009, 32(11), 1545–1554. https://doi.org/10.1007/s12272-009-2106-0.
49. Al-Asmari, A. K.; Riyasdeen, A.; Islam, M. Scorpion Venom Causes Apoptosis by Increasing Reactive Oxygen Species and Cell Cycle Arrest in MDA-MB-231 and HCT-8 Cancer Cell Lines. Journal of evidence-based integrative medicine, 2018, 23, 2156587217751796.
50. Franco, R.; Cidlowski, J. A. Apoptosis and glutathione: beyond an antioxidant. Cell death and differentiation, 2009, 16(10), 1303–1314.
51. Mandal, I.; Manna, S.; & Venkatramani, R. UV-Visible Lysine-Glutamate Dimer Excitations in Protein Charge Transfer Spectra: TDDFT Descriptions Using an Optimally Tuned CAM-B3LYP Functional. The journal of physical chemistry. 2019, B, 123(51), 10967–10979. https://doi.org/10.1021/acs.jpcb.9b07827.
52. Ghanghro, A.; Ul, N.; Qureshi, H.; Ghanghro, I.; Jahangir, T.; & Bhurgri, M.; Ahmed, Z.; & Ahmed, S. Sds-page profile and biochemical study on the venom of some medically important snakes of sindh. IJBPAS, 2016, 6808-6815.
53. Cho, Y.; Song, K. B. Effect of G-Irradiation on the Molecular Properties of BSA and b-Lactoglobulin. J. Biochem. Mol. Biol. 2000, 33, 133-137.
54. Minton, S.; Weinstein, S. Colubrid Snake Venoms: Immunologic Relationships, Electrophoretic Patterns. Copeia. 1987,. 993. 10.2307/1445563.
55. Bennacef-Heffar, N.; Laraba-Djebari, F. Evaluation of the effect of gamma rays on the venom of Vipera lebetina by biochemical study. Canadian journal of physiology and pharmacology, 2003, 81(12), 1110–1117. https://doi.org/10.1139/y03-112.
56. Abdel-Aty, A.M.; Salama, W. H.; Ali, A.A.; Mohamed, S.A. A Hemorrhagic Metalloprotease of Egyptian Cerastes Vipera Venom: Biochemical and Immunological Properties. Int. J. Biol. Macromol. 2019, 130, 695-704.
57. Chapelier, A.; Desmadril, M.; Houée-Levin, C. Gamma radiation effects on alpha-lactalbumin: structural modifications. Canadian journal of physiology and pharmacology, 2001, 79(2), 154–157. https://doi.org/10.1139/cjpp-79-2-154.
58. El-Yamany, M. F.; Samy, E. M.; Salama, W. H.; Shaaban, E. A.; Abd El-Latif, H. A. Gamma irradiated protease from Echis pyramidum venom: A promising immunogen to improve viper bites treatment. Toxicon : official journal of the

International Society on Toxinology, 2020, 188, 108–116. https://doi.org/10.1016/j.toxicon.2020.10.013
59. Abdel-Aty, A.M.; Wahby, A.F.. Purification and Characterization of Five Snake Venom Metalloproteinases from Egyptian Echis Pyramidum Venom. J. Toxicol. Sci. 2014, 39, 523-536
60. Dyab, A.; Ahmed, M.; Abdelazeem, A. Prevalence And Histopathology of Trichinella Spiralis Larvae Of Slaughtered Pigs In Cairo Governorate, Egypt. Journal of the Egyptian Society of Parasitology, 2019; 49(2): 439-442. doi: 10.21608/jesp.2019.68187.
61. Lee, Y.; Song, K. B. Effect of gamma-irradiation on the molecular properties of myoglobin. Journal of biochemistry and molecular biology, 2002, 35(6), 590–594. https://doi.org/10.5483/bmbrep.2002.35.6.590.