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Therapeutic effects of *Apis mellifera* and *Pandinus Imperator* venom on lung cancer: An *In vitro* study

Abir A. El-fiky[1], Gehan Hammad[2*], Mariam G. SaadEldin[2]and Omar H. Elhiatmy[2]

[1]Director of ANDI Center of Excellence in Antivenom Research, VACSERA, **Egypt**.
[2]Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), **Egypt**

***Correspondence:** gmhammad@msa.eun.eg **Accepted:** 10 July 2019 **Published online:** 03 Aug. 2019

Lung cancer persists as the leading cause of cancer incidences and mortalities worldwide, and is ranked 4th in Egypt. Globally, 2.1 million new lung cancer cases and 1.8 million deaths were reported in 2018, representing close to 1 in 5 (18.4%) cancer deaths. Venom components have been investigated for pharmacological activity as therapeutic agents. Intriguingly, several animal toxins have demonstrated profound anticancer properties. These include toxins purified from snake, bee and scorpion venoms effecting cancer cell proliferation, migration, invasion, apoptotic activity and neovascularization. In this study, *Apis mellifera* (bee venom) and *pandinus imperator* (Scorpion venom) were assessed in terms of anti-cancer potential in vitro, in comparison to Staurosporine (a potent protein kinase inhibitor) on Hs57T cell lines. The cytotoxic, cell cycle arrest, and apoptotic effects were evaluated. The results demonstrated IC_{50} values for *Apis mellifera* and *Pandinus imperator* as 2.42 & 1.054 µg/ml respectively. Both venoms exhibited significant apoptotic effects and cell cycle arrest potential at G2/M, as well as anti-tumor regulatory effects due EGFR, VEGFR2, and MMP-1 expression level decreased assessed via RT-qPCR in Hs57T cell lines.

Keywords: Lung Cancer, Hs57T (Sarcoma or Lymphoma), *Apis Mellifera*, *Pandinus Imperator*, apoptosis, anti-cancer effects, Staurosporine

INTRODUCTION

Worldwide, lung cancer remains the leading cause of cancer incidence and mortality, with 2.1 million new lung cancer cases and 1.8 million deaths in 2018, representing close to 1 in 5 (18.4%) cancer deaths (Bray et al., 2018). According to Globocan, 2018, Lung cancer is ranked 5th (4.7%) in terms of incidence, and 4th (6.6%) in terms of mortality in Egypt. In developed countries, technological advancements and enhanced awareness contribute to a decreased rate of mortality, when compared to previous years. In contrast, in Egypt, more effective diagnostic imaging techniques and awareness are required to decrease lung cancer mortality rates (Dubey et al., 2016). A national updated cancer registry is essential for enabling efficient strategies for cancer prevention, treatment, and control (Ferlay et al., 2010). Decreases in smoking have manifested as declines in lung cancer incidence rates (El-Bolkainy, et al., 2016). In regards to males' incidences the highest being in Western Asia, Northern Africa, Eastern Europe. Incidences are relatively lower in Africa. In females it represents the highest cause of cancer related deaths in 28 countries. The variation of lung cancer rates by region is approximately 20-fold, and reflected via the maturity of smoking epidemics and tobacco exposures, according to the duration of smoking, types and degrees on inhalation (Bray et al., 2018). El-Moselhy1 and El Rifai, (2018), report that over 80% of lung cancers are attributed to smoking, and that prevention through tobacco control measures, reduce active smoking and involuntary exposure to smoke. These measures are outlined and embedded

along with advertising within the WHO Framework convention on Tobacco Control (WHO, 2018; Stewart & Wild, 2014). Primary pleuropulmonary sarcoma is a heterogeneous type of tumor inferred from cells of mesenchymal origin, which seem to emerge from the lung and pleura. Essential chest divider sarcomas are uncommon, representing 0.2% of all lung cancers and 5% of all thoracic neoplasms (Levra et al., 2012). Lung cancer regularly shows up as a singular pneumonic knob on a chest radiograph. In any case, the differential conclusion is wide. Furthermore, numerous other illnesses favor more specific imaging, including counting metastatic cancer, hamartomas, and irresistible granulomas caused by tuberculosis, histoplasmosis or coccidioidomycosis (Ingbar, 2015). Confirmation of lung cancer is based on histological examination (Jameson, et al., 2018).

Animals' venom are secretions mostly utilized by the organisms for defense and/or immobilization of their prey (Fernandes-Pedrosa, et al., 2013). These secretions are mostly a complex mixture of biologically active components including neurotoxins (targeting nerve impulses' transmissions), pain inducing enzymes and substances (e.g. ion channel toxins), and mycotoxins (i.e. attack and break down muscle tissue) (Chaisakul, et al., 2016). Subsequently, the research associated with identifying lead compounds for novel therapeutics majorly represents the employment of animal venom. Therapeutics based on venom include, captopril (*Bothrops jararaca*), exenatide (*Heloderma suspectum*) and ziconotide (Conus magus). Advanced stages of lethal cancers such as lung remain a significant cause of mortality, due to their 'aggressiveness' i.e. their ability to grow and their metastases to other regions causing devastating outcomes (Ferlay, et al., 2015; Hanahan and Weinberg, 2011). The employment of particular strains of snake and Anthropos venom were previously evaluated for their selective toxicity on a variety of cancer cells (Ebrahim, et al., 2015; Dubovskii et al., 2014; Donato et al., 1996; Huh et al., 2012; Tseng, et al., 2004). Cura et al., 2002, demonstrated the activity of administrating crotoxin (a neurotoxin isolated from *Crotalus durissus terrificus* venom) for its an analgesic effect to advanced cancer patients which minimized their consumption of analgesics. Sweet bee venom was applied by Yoon, et al., 2012, as pharmaco-puncture for patients with chemotherapy-induced peripheral neuropathy. Several distinct components have previously exhibited effects on cancer development and could provide an alternative platform for clinical e.g. chlorotoxin, melittin, polybia-MPI, phospholipase A2, L-amino acid oxidase, disintegrins (Ma, et al., 2017; Chatterjee, 2018; Li, et al., 2018).

Apis mellifera or Bee Venom (BV), comprises melittin and several other natural dynamic peptides including apamin, mast cell degranulating (MCD) peptide, and adolapin, it also contains a complex blend of chemicals α-galactosidase, phospholipase A2 (PLA2), hyaluronidase, α-acetylamino-deosiglucosidase, hyaluronidase, and corrosive esterase, as well as nonpeptide components including norepinephrine, histamine, and dopamine (Son, et al., 2007; Raghuraman and Chattopadhyay, 2007; Fernandes-Pedrosa, et al., 2013). Over the past decade, several considerations were established to the radio protective, antinociceptive, anti-inflammatory, and anti-cancer potential of BV (Varanda, and Tavares, 2005; Son et al., 2007; Varanda, and Tavares, 1999; Fernandes-Pedrosa, et al., 2013). A development of research exhibited their impact for inducing apoptosis, affecting expansion, cytotoxicity and restraining development for several cancer cell types (Li, et al., 2018; Fernandes-Pedrosa, et al., 2013; Han, et al., 2007). Melittin has previously demonstrated calmodulin activity and inhibitory effects of growth and clonogenicity of human and murine leukemic cells (Ownby et al., 1997; Samel et al., 2013; Nam, et al., 2008). Previously, studies demonstrated inhibition of cell growth and induction of apoptosis in prostate and ovarian cancer cells (Park, et al., 2011; Jo, et al., 2011; Wang, et al., 2007). Moreover, the treatment demonstrated apoptotic cell death in both caspase-dependent and caspase-independent manners due to intracellular Ca (2+)-modulated intrinsic death pathway in human bladder cancer cells (Ip et al., 2012). However, there is little data for its effects in human lung cancer cells (Choi et al., 2014).

Venomous arthropods such as scorpions, possess a wide array of compounds in their venom including Gonearrestide, hyaluronidase, phopholipase, muco-polysaccharides, and relatively low molecular mass molecules such as histamine, serotonin, histamine releasers and polypeptidyl compounds (Fernandes-Pedrosa, et al., 2013; Rady, et al., 2017). Scorpion Venom (SV) is known for being a rich source of small neurotoxic proteins, which interact with ionic channels (Goudet, Chi, and Tytgat, 2002), the

most predominat being chlorotoxin (Cltx) (Olamendi-Portugal, et al., 1996). Soroceanu, Manning, and Sontheimer, 1999, demonstrated the binding potential of Cltx to glioma cells with little to no activity on normal cells. Several peptides e.g. neopladine 1 and neopladine 2, and Gonearrestide were purified from scorpion venom and found to be active against human breast carcinoma SKBR3 cells & colorectal cancer cells, HCT116, by binding to the cell surface (D'Suze, et al., 2010; Li, et al., 2018). Results indicating that scorpion venom could represent candidancy for the development new clinical treatments against tumors. Pandinus imperator (PI) is one of the most common species of scorpion worldwide, with two types of venom imperatoxin I and A(IpTx) and pandinotoxin (PiTX-Kα) (Rowe, 2004; Zamudio et al., 1997). The venoms demonstrated similar pathways of intracellular Ca (2+)-modulated intrinsic death by binding to ryanodine receptors (RyRs) (Shahbazzadeh et al., 2007; Duan et al., 2012).

In the current study, the in vitro anticancer activities of BV and PI were examined in a penal of human sarcoma and lymphoma cancer cell lines (Hs 57.T) as an alternative cancer treatment (i.e. lung cancer). The bioassays used to evaluate the anticancer activities included cytotoxicity assay, cell cycle arrest analysis and measure gene expression levels of three selected genes (EGFR, VEGFR2, and MMP-1).

MATERIALS AND METHODS

Materials

Cell culture Media and MTT staining, penicillin-streptomycin, 25% trypsin-EDTA, and propidium iodide DNA staining and all other reagents were all purchased from (Sigma Aldrich, USA). Cell culture grade dimethyl sulfoxide (DMSO), medium RPMI-1640, human lung cancer (Hs57T) cell line was kindly supplied from the R&D Sector, The International Center for Advanced Research, and previously acquired from the American Type Culture Collection (ATCC, Rockville, MD, USA). Scorpion venom (*Pandinus imperator* venom) (PI), bee venom (BV) and Staurosporine drug were provided from ANDI CO, VACSERA, Egypt) (abbreviation in parentheses) preliminary study showed the optimal dosages of venoms to be around 0.8 μg/mL, where cells appeared morphologically composed and not much cell destruction was observed. Taking into consideration this optimal dose, all experiments in this study were performed 2.42 and 1.054 ug/ml final concentration unless otherwise stated. Annexin V-FITC was from Bio-Vision Research Products, USA and iScript TM one-step real time-PCR Kit with SYBR® Green was from Bio-Rad Laboratories, USA. One mg of each venom was diluted in 1mL phosphate buffer saline (1mg/1mL). All materials were serially diluted in RPMI-1640 media to the appropriate concentrations (Gajski et al., 2014).

Maintenance of cell line and culture conditions

The Hs57T (human lung sarcoma cell line) was provided by VACSERA-Cell Culture Unit. Cells were cultured in RPMI medium in the T-75 flasks (Griener, Germany) supplemented with 10% fetal bovine serum. The cell cultures were maintained at 37ºC, 95% humidified atmosphere with 5% CO_2. Monolayer cells were trypsinized at 80% confluency (Masters, 2000). Cell counts: accurate cell numbers in suspension were calculated using a hemocytometer (Soliman et al, 2013). Briefly, double fold dilution of the original cell suspension was prepared by adding 0.5mL of the undiluted cell suspension to 0.5mL of 0.4% trypan blue dye. They were then mixed well and immediately aspirated to fill the hemocytometer counting chambers.

Cytotoxicity using MTT assay

Cells were plated in 96-well-plates with 100μL of RPMI medium. 200μL were serially diluted twice. The plates were then incubated with the various venom concentrations for 24hr at 37ºC. For the MTT assay, 96-well-plates were washed with PBS and 50μL of MTT were added into each well, and then incubated for 3hr at 37ºC. 50μL of isopropanol were added and incubated for 5 minutes at 37ºC. Plates were read using a micro-plate reader at 570nm wavelength, and absorbance was correlated with the respective cell number (Marí-Beffa et al., 2007). Cytotoxic effects of tested venoms on the Hs57T cell lines were expressed as the IC_{50} values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells). Inhibitory concentration (IC_{50}) of compounds were assessed using Masterplex software (2010). Cell survival rate was calculated as (absorbance of the treated wells)/ (absorbance of the control wells) × 100%.

Cell cycle analysis using propidium iodide DNA staining by Flow Cytometry:

Cells were harvested in an appropriate manner and washed with PBS, then fixed in cold.

Table 1: Primer sequences for RT-qPCR for gene expression assessment as generated from Rotor-Gene 6000 Series Software 1.7:

Primers	Forward	Reverse
EGFR	5’-GAGAGGAGAACTGCCAGAA-3’	5’-GTAGCATTTATGGAGAGTG-3’
VEGFR2	5’-GTGACCAACATGGAGTCGTG-3’	5’-CCAGAGATTCCATGCCACTT-3’
MMP1	5’-AGCTAGCTCAGGATGACATTGATG-3’	5’-GCCGATGGGCTGGACAG-3’

70% ethanol. While, vortexing, the pellet was dissolved drop-wise.

The cells were fixed for 30min at 4ºC and minimized clumping. After ensuring fixation, cells were washed in PBS and spun at 850g. The supernatant was discarded and the cells were treated with ribonuclease I (from 100mg/ml stock solution). 50μl of 100μg/ml stock of RNase was added to ensure only DNA (not RNA) was stained. Then, 200μl propidium iodide was added (from 50μg/ml stock solution) (Davies, 2012). Apoptotic detection using Annexin V-FITC assay by Flow Cytometry: 1-5x10, 5 cells were collected by centrifugation then suspended in 500μl of 1X binding buffer. 5μl of Annexin V-FITC & 5μl of propidium iodide (PI 50mg/ml) were added. Cells were incubated at room temperature for 5min in a dark room. Annexin V-FITC binding was analyzed by flow cytometry (Ex= 488nm; Em= 530nm) by FITC signal detector & PI staining by the phycoerythrin emission signal detector. For adherent cells, gently trypsinized and washed once with serum-containing media before incubation with Annexin V-FITC (Bio-Vision Research Products). The cells were washed twice with cold phosphate-buffered saline (PBS) and 200 μL of Annexin-V binding buffer, 10 μL of Annexin-V-FITC and 10 μL of PI working solution were added. After incubation in the dark for 15 min at room temperature the reaction mixture was removed and the cells were washed with Annexin-V binding buffer. Then, the cover slips with cells were taken out from the wells and the mounted preparations were made. The viability of the treated and non-treated (control) cells was observed under an epifluorescence microscope.

Gene expression analysis after treatment with venoms and Tamoxifen:

The expression of regulatory and apoptotic genes (EGFR, VEFGR2, and MMP-1) were examined using real-time PCR in Hs57T cell lines. Primer sequences of oligonucleotides used for real time-PCR shown in the following table. Total RNA was extracted from both treated and untreated cells 24h post-treatment by iScript TM One-Step real time-PCR Kit with SYBR® Green (Romeiro, et al., 2016) by real-time quantitative-PCR of RNA templates. cDNA synthesis and PCR amplification were carried out routinely. Melting curve analysis of PCR products was performed by Step One TM Real-Time Systems software (Kit, 2012). Primer sequences for gene expression analysis were generated via a Rotor-Gene 6000 Series Software 1.7 (Build 87) Copyright ©2000-2006 Corbett Research, a Division of Corbett Life Science. All rights reserved. ISO 9001:2000 (Reg. No. QEC21313)

Statistical analysis:

Data was processed statistically according to Snedecor and Cochran (1982), where minimum, maximum, mean value, standard deviation, standard error, and range were presented. Mean values ± standard deviation (SD) of absorbances obtained from cells incubated in the presence of the treatments, were calculated. Results were expressed as the percentage of the corresponding negative control conducted in the same experiment. Statistical analysis was performed using GraphPad Prism 6 software. P values were calculated by student’s t-test from the mean values of the indicated data. Significant differences were marked with asterisks (*P < .05; **P < .01; ***P < .001; ****P < .0001).

RESULTS

Inhibitory effects of BV, PI on Cancer Cell viability:

Cancer cell lines (Hs 57T) were exposed to BV & PI at standard concentrations. The results are shown in Table 2 and Figure 1. There were significant inhibitory effects of studied preparations towards Hs 57T cells in their concentration range; Figure 1(A): demonstrates the collective means, showing the inhibitory effects of BV & SCV; SCV demonstrated the higher inhibitory effects and cytotoxicity values i.e. potency, followed by BV when compared to the standard drug, which demonstrated the highest effects.

Table 2: Tabulation of BV, PI and standard anticancer drug Staurosporine, Table shows Mean IC_{50} ± SD and molecular weights

Series	Sample code	Molecular Weight	Mean IC_{50} values ± SD □g/ml
1	BV/HS57T	2846.515 g/mol	2.42±0.082***
2	SCV/HS57T	3,765 Da	1.054±0.051***
3	Staurosporine	466.53 g/mol	14.23±1.52

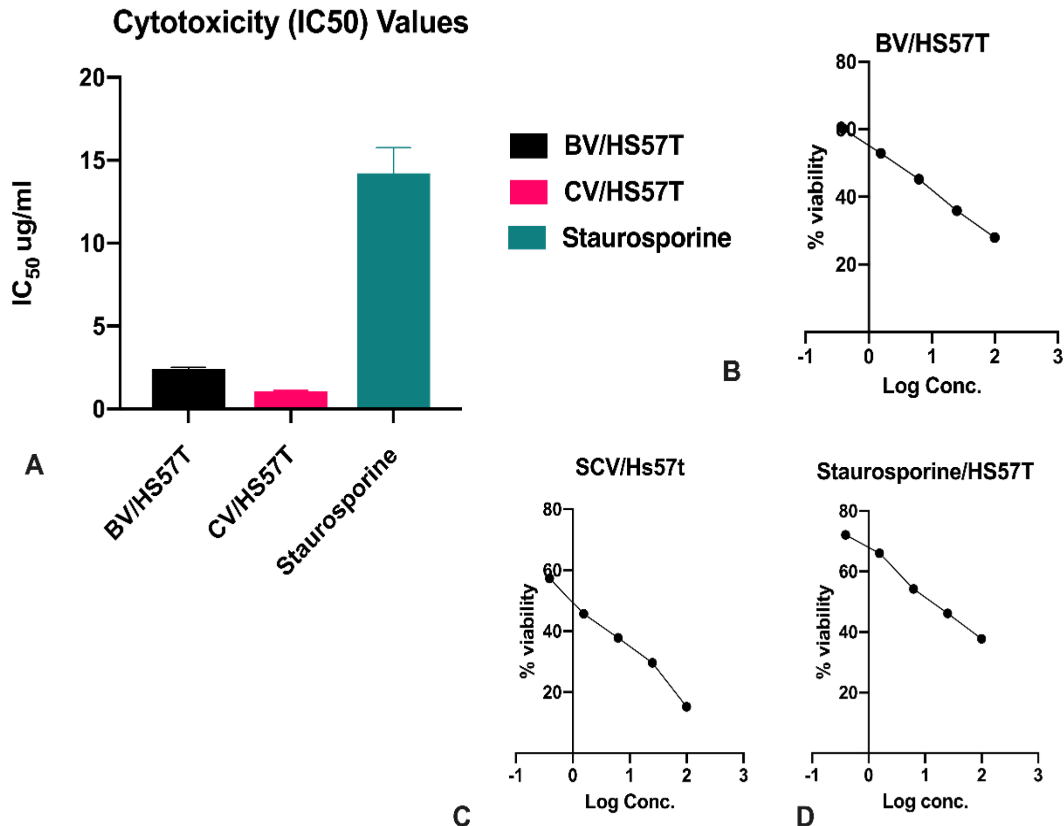

Figure 1: (A) Graphic representation of the summarized cytotoxic effects Cytotoxicity BV, PI and standard staurosporine, After treatment for 24 h, the viability of the cells was determined by MTT assay Data are means (±SD) from two independent experiments performed in triplicates; (B) Values for viability percentages transformed log concentrations to demonstrate viability when treated with SCV; (C) Values for viability percentages transformed log concentrations to demonstrate viability when treated with BV; (D) Values for viability percentages transformed log concentrations to demonstrate viability when treated with anticancer drug Staurosporine.

Cell Cycle arrest effects of of BV, PI on Cell viability by flowcytometery using Annexin-V FITC:

Annexin V and PI staining followed by flow cytometry can differentiate cells into different stages. The results of these experiments were shown in Figure 2 (A-J). Cell cycle phase distribution of cells treated with/without venoms were shown in Figure 3A and Table 3. The data demonstrated that cancer cell cycle was downregulated in G2/M phase, and was consistent with our findings that showed the cell cycle signals were affected after treatment.

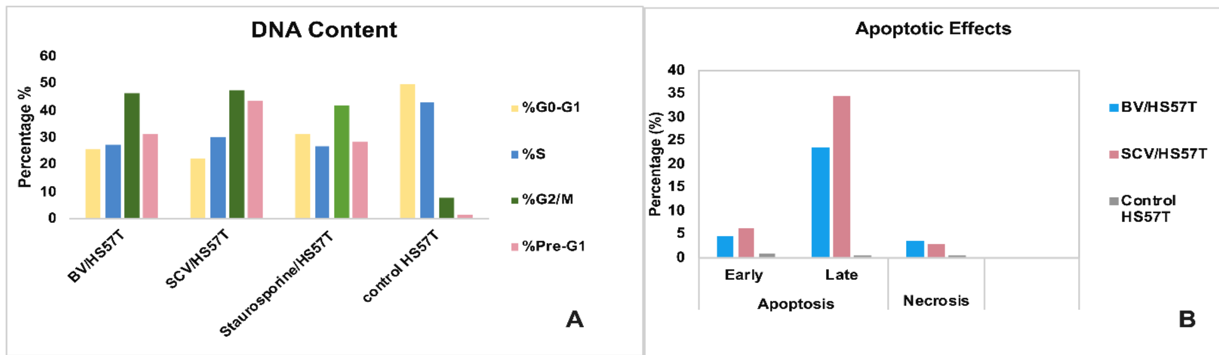

Figure 2: (A) Summary Percentages of cell cycle arrest effects using flow cytometery; (B) Summary Percentages of apoptotic effects results using flow cytometery; (C-J). Flow cytometry results using Annexin-V FITC using Diploid and PI on BV, SCV, Staurosporine and non-treated Hs57T cancer cell lines.

Table 3: Tabulated report of BV, PI and standard anticancer drug Staurosporine when treated with Hs 57T cells and their respective control, in terms of Cell Cycle Arrest effects:

Sample data		Results				
S/N	Code	%G0-G1	%S	%G2/M	%Pre-G1	Comments
1	BV/HS57T	25.81	27.59	46.6*	31.47	Pre-G1 apoptosis & Cell growth arrest @G2/M
2	SCV/HS57T	22.49	30.28	47.23*	43.59*	Pre-G1 apoptosis & Cell growth arrest @G2/M
4	Staurosporine /HS57T	31.25	26.82	41.93	28.72	Pre-G1 apoptosis & Cell growth arrest @G2/M
3	Control HS57T	49.57	42.76	7.67	1.82	

*Significant differences marked with asterisks (*P < 0.05).*

Table 4: Tabulated report of BV, PI and standard anticancer drug Staurosporinewhen treated with Hs 57T cells and their respective control, in terms of Apoptotic and Necrotic effects:

S/N		Apoptosis			Necrosis
		Total	Early	Late	
1	BV/HS57T	31.47	4.66*	23.4	3.41
2	SCV/HS57T	43.59*	6.12*	34.66**	2.81
3	Control HS57T	1.82	0.91	0.36	0.55

*Significant differences marked with asterisks (*P < .05; **P < 0.01).*

Table 5: Tabulated report of gene expression results in terms of fold change 2^ ΔΔCT for BV, SCV, and Staurosporine treated Hs57T Cancer cell lines, and the untreated controls:

Sample data		Gene expression Fold Change (2^ ΔΔCT)		
		HS57T		
S/N	Sample code	EGFR	MMP1	VEGFR2
1	BV/HS57T	0.172086**	0.437679	0.429475
2	SCV/HS57T	0.110661**	0.120117*	0.288645*
3	Staurosporine/HS57T	0.159541**	0.238884	0.496525
4	control HS57T	1	1	1

*Significant differences marked with asterisks (*P < .05; **P < 0.01).*

The report genertated demonstrated the following percentages: For BV they were %G0-G1: 25.81; %S 27.59; %G2/M: 46.6; and %Pre-G1: 31.47, indicating Pre-G apoptosis & Cell growth arrest at G2/M. For SCV (PI) they were %G0-G1: 22.49; %S 30.28; %G2/M 47.23; and %Pre-G1 43.59 also indicating Pre-G apoptosis & Cell growth arrest at G2/M.

Apoptotic effects of of BV& PI on Cell viability by flowcytometery using Annexin-V FITC :

Annexin V and PI staining and flow cytometry can separate and identify cells into different stages. The results of these experiments were shown in Figure 2 (A-J) and Table 4. The data showed that induced apoptosis of the cancer cells when compared to blank controls—a finding which was also consistent with RNA expression analysis of this study i.e. downregulation of selected genes. The report genertated demonstrated the following percentages: For BV & SCV respectively: total apoptosis (31.47; 43.59*) early apoptosis (4.66*; 6.12*) Late apoptosis (23.4; 34.66**).

Gene expression Analysis effects for BV& PI:

To assess the effect of the treatments, the expression of EGFR, MMP1, and VEGFR1 were conducted to determine the effects of the potential therapeutics, the results demonstrated a downregulation in all of the expressed genes, but only EGFR was statistically significant (*P< 0.01*); SCV demonstrated an impact of significant downregulation (*P< 0.05*) (Table 5 and Figure 3).

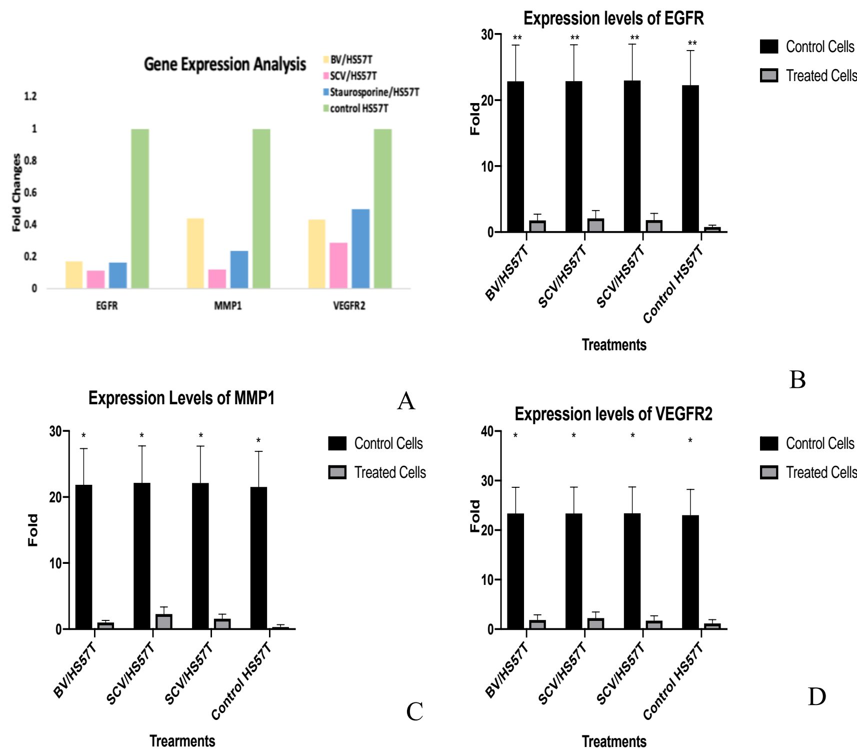

Figure 3: (A) Summary of gene expression analysis using real-time PCR, through fold change calculations; (B) EGFR gene expression experiment, significant downregulation after treatment; (C) MMP1 gene expression experiment, significant downregulation after treatment on Cancer Cells; (D) VEGFR2 gene expression significant down regulation after

DISCUSSION

The utilization of animal venom has demonstrated success in terms of illustrating its potential as a therapeutic against several diseases (Putz, et al., 2006; Chu, et al., 2007). This is particularly true for venom-based peptide/toxins, and investigations related to them have provided insights towards various diagnostic and therapeutic possibilities over the past decade. Venom from animals has been proven to be a useful source of drug candidates in natural drug discovery to fight against diseases ((Putz, et al, 2006; Chu, et al., 2007). Especially for venom-based peptide/toxin, it has paved new insights into therapeutic and diagnostic potential for cancer treatment in the last decade (Gajski, et al., 2013; Qian, et al., 2015; Huh, et al., 2012; Liu, et al., 2008).

Chaisakul, et al., 2016, discussed the effects of venoms and toxins on the hallmarks of cancer. Recent studies indicated that some of these venomous peptide molecules with membrane receptor molecules, extracellular matrix, and non-receptor components, which could stimulate the inhibition or dyregulation of a hallmark as expressed by Hanahan & Weinberg, 2011; Wang, et al., 2015).

Examples of these effects was the NN-32, from Naja naja venom, which expressed cytotoxic effects on MDA-MB-231 and MCF-7 cells, which induced direct cancer death Qian, et al., 2015. Other examples were sPLA2 and WEV, purified from *Vipera ammodytes meriodionalis*, and *Walterinnesia aegyptia*, respectively, which provided apoptotic effects on a wide range of cancer cell lines. Additionally, WEV and Silica nanoparticles effectively which enhanced the suppressive effects in mouse models in vitro Qian, et al., 2015; Huh, et al., 2012; Kim, et al., 2015; Cogulu, et al., 2009). Finally, Al-Asmari, et al., 2017 and Al-Asmari, et al., 2018, encapsulated scorpion venoms, which exhibit better efficacy and acted more vigorously as an anti-cancer agent on colorectal cancer cell line when compared with their free counterpart. Scorpion venom has demonstrated therapeutic potential against various cancers (Gomes, et al., 2009; Heinen and Veiga, 2011) The utilization of an encapsulation form for the tested materials could be a potential enhancement of the cytotoxicity and anti-cancer outcomes of this study. Lung cancer is a major affliction that constantly requires review of novel therapeutic platforms. Zhang, et al., 2005 has previously noted the potentially powerful anti-cancer effects of scorpion venom from *pandinus imperator* on androgen independent lung cancer cell lines. Oršolić, 2012; Son et al., 2007; Fernandes-Pedrosa, et al., 2013, all highlighted the impacts of bee venom in terms of apoptotic, necrotic, cytotoxic, and inhibitory effects on cancer cells. The effects were restricted towards hindering cancer cell and tumor development, whilst maintaining little to no effects on normal cells.

In the present study, the anti-cancer effects of PI and BV were evaluated on Hs57T cell lines, in comparison to a common anti-cancer drug Staurosporine. The evaluated cytotoxicity assessments were consistent with the work of Zaky, et al., 2015, who highlighted IC_{50} values of bee venom on MCF7 cell lines, which provided a value of 2.85 µg/mL. This value is almost equivalent to the evaluated IC_{50} value of bee venom on HS57T cell line after 4 hours of incubation acquired from this study which was found to be 2.42µg/mL. Conversely, Sisakht et al., 2017 and El-fiky et al., 2017, presented IC_{50} values of 28.52 µg/mL & 4.1 µg/mL on human glioblastoma and A549 cell lines respectively. Staurosporine is a known non-specific inhibitor of protein kinases, and when targeted effects for cervical carcinogenesis in terms of antitumor activity is known e.g. Yadav, et al., 2015, estimated its potential on cervical cancer cell lines, and found potent potential. According to Slightly, *et al.,* 1986. IC_{50} of Staurosporine on Pfpkf is 1mM. In the present study, the IC_{50} value of (Hs57T) treated with Staurosporine was 14.23µg/mL, while the IC_{50} value from *pandinus imperator* venom was 1.054 µg/mL. These do not coincide with the results of Klenk, et al., 2000, but work with the results of Li, et al., 2018, these differences in presentation or potency could be mainly attributed to the potential of cell lines, which concluded that the highest cytotoxic effects were present for SCV, followed by BV then Staurosporine, however encapsulation could provide better prospects.

The Cell cycle arrest & apoptotic effects of the assessed venoms on the Hs57T cancer cell lines were evaluated using flowcytometry. In regards to cell cycle arrest statistical significance ($p<0.05$) was observed and demonstrated efficacious cell cycle arrest at G2/M for both BV and PI. It seems as though cell cycle effects and apoptotic cell passaging at both preG1 and G2/M was observed, which was correlated to the findings of Alizadehnohi et al., 2017, where BV was applied on human lung A549 cells, and exhibited preG1 and G2/M and cell cycle arrest and apoptosis.

Pandinus imperatr (PI) showed the same findings, which was confirmed by Ding et al., 2014 and Li, et al., 2018, who postulated that the dynamic particles possess anticancer activity that are consistent with the insightful review of Heinen & Veiga, 2011, including antiproliferative, dysregulation of the cell cycle, induction of apoptosis, and decreased motility and development. These were presented for SVs and BVs on a multitude of cancer cells including glioma, neuroblastoma, leukemia, lymphoma, breast, lung and prostate cancers. Similar outcomes were presented with Staurosporine on the cell cycle HS57T cell line, pre G1 apoptosis and cell cycle arrest at G2/M this comes in assertion with Tamaoki et al.,1991; Yadav, et al., 2015, both of which explored the kinase inhibitor's anti-tumor activity in vitro & in vivo and expressed similar findings. Our findings indicated late apoptosis and necrosis on all assessed materials. These correlated to Abir, et al., 2018, on HepG2 cell lines treated with bee venom. But not Alizadehnohi, et al., 2012, who exhibited early apoptotic effects with bee venom on ovarian cancer cells A2780cp. Dardevet et al., 2015, postulated late apoptosis as well as necrosis with Staurosporine, which correlate with our experiments. Furthermore, Melanoma treated with staurosporine showed late apoptosis as well as necrosis, Contrariwise, the apoptotic effects of staurosporine on a human corneal endothelial cells were at early stages (Thuret et al., 2003). The antiproliferative effects demonstrated through the downregulation of the cell cycle, and the induction of late apoptosis is a powerful indicator of the antitumor effects of both Bee Venom, and Scorpion Venom treatments, however, the cytotoxic effects were mostly witnessed for PI, this would suggest encapsulation for delivery as an enhancement to the treatments to strengthen its potency and therapeutic potential as anticancer drugs for a devastating affliction as lung cancer, which would be the future direction for this study.

The role of growth factors-driven signaling in the pathogenesis of human cancer progression has been extensively investigated (Chetty, et al., 2010). It has been previously determined that cancer cells rely on autocrine secretion of growth factors, and reduce their dependency on exogenous growth factor supplementation for signaling proliferation (Hanahan, et al. 1996). The epidermal growth factor receptor (EGFR) is often heavily involved in the pathogenesis and progression of different carcinoma types. EGFR is described to occur on average at 50% to 70% in the majority of human carcinomas including lung, colon and breast carcinomas. Similarly, VEGF and its receptors (VEGFRs) are key modulators of a known hallmark of cancer: angiogenesis; and specifically, was found to increase the proliferation of endothelial cells from neighboring blood vessels through interactions with VEGFR-2 (Seto, et al., 2006; Mercurio, 2004). VEGFR2 functions in regulating angiogenesis, promoting proliferation, and migration survival, as well as previously demonstrating a role in the differentiation of endothelial cells, thus providing it with a part in metastasis (Shibuya, 2006). Subsequently, its downregulation was linked to tumor regression. MMP-1, a neutral pH interstitial collagenase enzyme, which participates in tissue breakdown during tumor development, and has been linked to several lung tumors Pendás et al., 1996. Due to the aforementioned statements, these genes were selected for the current study, gene expression was assessed for all treatments on the Hs57t cancer cell line.

In case of Staurosporine treatments the results were that the gene expression of EGFR decreased 6.3-fold, MMP1 was downregulated by 4.2-fold, VEGFR2, meaning it was also downregulated by 2-fold. The changes in gene expression justify the anti-proliferative effects of the kinase inhibitor, while highlighting the mechanism which distances itself from cytotoxicity results. These results agree with Leu, et al, 2000 and Yadav, et al., 2015, where Staurosporine stimulates apoptosis by inducing MCL-1 via the mitogen-activated protein kinase pathway.

Regarding Bee Venom, nearly the same outcomes of Staurosporine treatments was observed, approximately 6-fold decrease in expression for EGFR. 2.3-Fold downregulation of MMP1 expression, and 2.3-Fold decrease in the expression of VEGFR2. Putz, et al., 2007, Presented similar outcomes in regards to EGFR gene expression, which attributes this dysregulation to a block in the forcibly induced sustained proliferative signals, and was also associated with another hallmark which was immune destruction (Lindsey & Langhans, 2015). The results including treatments while using PI venom included for EGFR, meaning expression decreased by 9-fold, MMP1 also presented denoted downregulation by 8.3-fold, and finally VEGFR2 was downregulated by 3.5-fold. Han, et al., 2007, expressed the presentation of MMP-1 and MMP-3's inhibitory effects against ultraviolet B induced human dermal fibroblasts. Ashutosh, et al., 2012 proclaimed MMP-1's induction of

apoptosis in cultured primary human neurons, both of which presented expression effects similar to the ones reviewed in this study. Furthermore, Huh, et al., 2012, examined similar outcomes in the assessment of anti-tumor effects of bee venom, and noted that the mechanism of action for anti-cancer activity in BV functions by VEGFR2 and COX-2 mediated MAPK signaling pathway. Furthermore, El-Miligy, et al., 2017, expressed that interest in targeting the suppression of VEGFR2 expression, should be a main focus for the inhibition of cancer growth and metastasis. Our results revealed significant anticancer effects of *pandinus imperator* on HS57T cells, through extensive downregulation of EGFR, MMP-1 and VEGFR2, that surpasses the action of the kinase inhibitor Staurosporine, with the additional protentional of decreasing metastatic effects as shown from significant downregulation of MMP-1 and EGFR (Collisson et al., 2014).

Taking all the results into consideration, the results for PI venom could be rationalized as follows, it possibly consists of two fractions Imperatoxin and pandinotoxin. Imperatoxin possesses nearly the same mechanism as a Cl- channel blocker as chlorotoxin from *Leiurus quinquestriatus* venom (DeBin, et al., 2017). CTX can specifically bind to the Cl- channel on glioma cells and inhibit glioma progression with evidence of electrophysiological properties, and subsequently expressed potential insights when applying recombinant His-CTX, which demonstrated the role of matrix metalloproteinase-2 MMP2 that was overexpressed on the glioma-cell surfaces (Lyons et al., 2002; Deshane et al., 2003).

The anticancer effects of venoms and toxins of scorpions *in vitro* and *in vivo,* were expressed through actions of inhibiting cancer growth, arresting the cell cycle, induction of apoptosis and suppression of cancer metastasis (Zhang & Zhang, 2016; Fernandes-Pedrosa, et al., 2013). This isn't solely attributed to the whole venom itself. Zhang et al., 2009, polypeptide extracts from the scorpion poison (PESV), of approximately 50-60 amino acids purified from BmK venom, affected cellular proliferation, and possessed apoptotic effects on DU 145 human prostate cancer cells.

CONCLUSION

From the previous results, *Apis mellifera venom* and *pandinus imperator* venom have an anticancer effect on lung sarcoma (HS75T cells) through exhibiting a late apoptotic effect that causes cell cycle arrest at G2/M in all tested compounds. Regarding gene expression levels of EGFR, VEGFR2 and MMP-1, *Apis mellifera* did not present a significant downregulation apart from EGFR, while *Pandinus imperator* venom significantly downregulated the gene expression levels. The mechanisms of these compounds in terms of anticancer effects are not fully understood, and perhaps a recommended enhancement for these results would be the use of a delivery system such as a nano-liposomal delivery for the venom to further enhance the outcomes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

A.F. and O.H. designed and performed the experiments, and prepared the methods and results section, G.H. & M.G., reviewed the sections, prepared the review of literature, the manuscript and results presentation.

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REFERENCES

Abdel-Aziz S. A. A, Mohamed A. F, Zahkouk S. A. M and Ali R. A. M. (2017); Evaluation of Anticancer Activity of Some Venomous Animal Toxins on Human Breast and Colon Cancer Cell Lines and Related Antioxidant Profile Int. J. of Adv. Res. 5 (2). 2036-2053] (ISSN 2320-5407).

Al-Asmari, A. K., Riyasdeen, A., & Islam, M. (2018). Scorpion Venom Causes

Upregulation of p53 and Downregulation of Bcl-xL and BID Protein Expression by Modulating Signaling Proteins Erk1/2 and STAT3, and DNA Damage in Breast and Colorectal Cancer Cell Lines. Integrative Cancer Therapies, 17(2), 271–281.

Al-Asmari, A. K., Ullah, Z., Balowi, A. Al, & Islam, M. (2017). In vitro determination of the efficacy of scorpion venoms as anti-cancer agents against colorectal cancer cells: A nano-liposomal delivery approach. International Journal of Nanomedicine, 12, 559–574.

Alizadehnohi, M., Nabiuni, M., Nazari, Z., Safaeinejad, Z., & Irian, S. (2012). The synergistic cytotoxic effect of cisplatin and honey bee venom on human ovarian cancer cell line A2780cp. Journal of Venom Research, 3, 22–27.

Ashutosh, Chao, C., Borgmann, K., Brew, K., & Ghorpade, A. (2012). Tissue inhibitor of metalloproteinases-1 protects human neurons from staurosporine and HIV-1-induced apoptosis: Mechanisms and relevance to HIV-1-associated dementia. Cell Death and Disease, 3(6).

Béchohra, L., Laraba-Djebari, F., & Hammoudi-Triki, D. (2016). Cytotoxic activity of Androctonus australis hector venom and its toxic fractions on human lung cancer cell line. Journal of Venomous Animals and Toxins Including Tropical Diseases, 22(1), 1–15.

Berger, E. (2017). Key Statistics for Lung Cancer. American Cancer Society. Retrieved from https://www.cancer.org/cancer/non-small-cell-lung-cancer/about/key-statistics.html

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.

Chaisakul, J., Hodgson, W. C., Kuruppu, S., & Prasongsook, N. (2016). Effects of animal venoms and toxins on hallmarks of cancer. Journal of Cancer, 7(11), 1571–1578. https://doi.org/10.7150/jca.15309

Chatterjee, B. (2018). Animal Venoms have Potential to Treat Cancer. Current topics in medicinal chemistry, 18(30), 2555-2566.

Chetty, C., Lakka, S. S., Bhoopathi, P. and Rao, J. S. (2010), MMP-2 alters VEGF expression via αVβ3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. Int. J. Cancer, 127: 1081-1095.

Choi, K. E., Hwang, C. J., Gu, S. M., Park, M. H., Kim, J. H., Park, J. H., Hong, J. T. (2014). Cancer cell growth inhibitory effect of bee venom via increase of death receptor 3 expression and inactivation of NF-kappa B in NSCLC cells. Toxins, 6(8), 2210–2228.

Choi, S. H., Yang, H., Lee, S. H., Ki, J. H., Nam, D. H., & Yoo, H. Y. (2014). TopBP1 and Claspin contribute to the radioresistance of lung cancer brain metastases. Molecular cancer, 13(1), 211.

Chu, S. T., Cheng, H., H, Huang, C., J., Chang, H., C., Chi, C., C., Su, H., H. (2007). Phospholipase A2-independent Ca2+ entry and subsequent apoptosis induced by melittin in human MG63 osteosarcoma cells. Life Sci, 80, 364-9.

Cogulu, O., Biray, C., Gunduz, C., Karaca, E., Aksoylar, S., Sorkun, K, (2009). Effects of Manisa propolis on telomerase activity in leukemia cells obtained from the bone marrow of leukemia patients. Int J Food Sci Nutrition. 60. 601-5.

Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: diagnosis and management. Am Fam Physician, 75(1), 56-63.

Collisson, E. A., Campbell, J. D., Brooks, A. N., Berger, A. H., Lee, W., Chmielecki, J., Cheney, R. (2014). Comprehensive molecular profiling of lung adenocarcinoma: The cancer genome atlas research network. Nature, 511(7511), 543–550.

Cura, J. E., Blanzaco, D. P., Brisson, C., Cura, M. A., Cabrol, R., Larrateguy, L., & de Roodt, A. R. (2002). Phase I and pharmacokinetics study of crotoxin (cytotoxic PLA2, NSC-624244) in patients with advanced cancer. Clinical Cancer Research, 8(4), 1033-1041.

D'Suze, G., Rosales, A., Salazar, V., & Sevcik, C. (2010). Apoptogenic peptides from Tityus discrepans scorpion venom acting against the SKBR3 breast cancer cell line. Toxicon, 56(8), 1497-1505.

Dardevet, L., Rani, D., El Aziz, T. A., Bazin, I., Sabatier, J. M., Fadl, M., De Waard, M. (2015). Chlorotoxin: A helpful natural scorpion peptide to diagnose glioma and fight tumor invasion. Toxins, 7(4), 1079–1101. https://doi.org/10.3390/toxins7041079

Davies, D. (2012) Flow cytomeric analysis of cell cycle with propidium iodine DNA staining,

Abcam. Retrieved from: [https://www.abcam.com/protocols/flow-cytometric-analysis-of-cell-cycle-propidium-iodine-dna-staining].

DeBin, J. A., Maggio, J. E., & Strichartz, G. R. (2017). Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. American Journal of Physiology-Cell Physiology, 264(2), C361–C369.

Deshane, J., Garner, C. C., & Sontheimer, H. (2003). Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. Journal of Biological Chemistry, 278(6), 4135–4144. https://doi.org/10.1074/jbc.M205662200

Ding, J., Chua, P. J., Bay, B. H., & Gopalakrishnakone, P. (2014). Scorpion venoms as a potential source of novel cancer therapeutic compounds. Experimental Biology and Medicine, 239(4), 387–393.

Donato, N. J., Martin, C. A., Perez, M., Newman, R. A., Vidal, J. C., & Etcheverry, M. (1996). Regulation of epidermal growth factor receptor activity by crotoxin, a snake venom phospholipase A2 toxin: a novel growth inhibitory mechanism. Biochemical pharmacology, 51(11), 1535-1543.

Duan, K. Z., Xu, Q., Zhang, X. M., Zhao, Z. Q., Mei, Y. A., & Zhang, Y. Q. (2012). Targeting A-type K + channels in primary sensory neurons for bone cancer pain in a rat model. Pain, 153(3), 562–574.

Dubey, A. K., Gupta, U., & Jain, S. (2016). Epidemiology of lung cancer and approaches for its prediction: a systematic review and analysis. Chinese journal of cancer, 35(1), 71. doi:10.1186/s40880-016-0135-x

Eberhard, W. G. (2004). The Encyclopedia of Land Invertebrate Behaviour. Rod Preston-Mafham, Ken Preston-Mafham. The Quarterly Review of Biology, 69(4), 559–560.

Ebrahim, K., Shirazi, F. H., Mirakabadi, A. Z., & Vatanpour, H. (2015). Cobra venom cytotoxins; apoptotic or necrotic agents? Toxicon, 108, 134-140.

El-Bolkainy, et al., (2016). Pathology of cancer. Retrieved from http://www.elbolkainy.net/wp-content/uploads/2016/03/2-Epidemiology-of-Cancer-15-310.pdf

El-fiky, A. A., Mohamed, A. F., Ismail, E. A., & Hirzi, H. (2017). Effect of Bee Venom and Other Natural Product (Dates) Onlung Cancer: Invitro Trial, 6(6), 2252–2257. https://doi.org/10.21275/ART20174874

El-Miligy, M. M. M., Abd El Razik, H. A., & Abu-Serie, M. M. (2017). Synthesis of piperazine-based thiazolidinones as VEGFR2 tyrosine kinase inhibitors inducing apoptosis. Future Medicinal Chemistry, 9(15), 1709–1729.

El-moselhy, E A; Elrifai, A. W. (2018). Risk Factors of Lung Cancer Worldwide and in Egypt: Current Situation | Insight Medical Publishing. Journal of Oncopathology and Clinical Research, 2(2), 5.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer, 127(12), 2893-2917.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer, 136(5), E359-E386.

Fernandes-Pedrosa, M. F., Félix-Silva, J., & Menezes, Y. A. (2013). Toxins from venomous animals: gene cloning, protein expression and biotechnological applications. In An Integrated View of the Molecular Recognition and Toxinology-From Analytical Procedures to Biomedical Applications. IntechOpen.

Fouda, S., Kelany, M., Moustafa, N., Abushouk, A. I., Hassane, A., Sleem, A., Bassiony, M. (2018). Tobacco smoking in Egypt: A scoping literature review of its epidemiology and control measures. Eastern Mediterranean Health Journal, 24(2), 198–215.

Gajski, G., Čimbora-Zovko, T., Rak, S., Rožman, M., Osmak, M., & Garaj-Vrhovac, V. (2014). Combined antitumor effects of bee venom and cisplatin on human cervical and laryngeal carcinoma cells and their drug resistant sublines. Journal of Applied Toxicology, 34(12), 1332-1341.

Gajski, G., Garaj-Vrhovac, V. (2013). Melittin: a lytic peptide with anticancer properties. Environ Toxicol Pharmacol. 36, 697-705.

Giaj Levra, M., Novello, S., Scagliotti, G. V., Papotti, M., & Le Cesne, A. (2012). Primary pleuropulmonary sarcoma: A rare disease entity. Clinical Lung Cancer. https://doi.org/10.1016/j.cllc.2012.05.003

Globocan-WHO (2019). Lung. World Health Organization. International Agency for Research on Cancer (IARC) [online] Available at:

http://gco.iarc.fr/today/data/factsheets/cancers/15-Lung-fact-sheet.pdf [Accessed 29 May 2019]

Gomes, A., Haldar, S., Giri, B., Mishra, R., Saha, A., Dasgupta, S., & Gomes, A. (2009). Experimental osteoporosis induced in female albino rats and its antagonism by Indian black scorpion (Heterometrus bengalensis C.L. Koch) venom. Toxicon, 53(1), 60–68.

Han, J. M., Lee, W. S., Kim, J. R., Son, J., Nam, K. H., Choi, S. C., & Jeong, T. S. (2007). Effects of diarylheptanoids on the tumor necrosis factor-α-induced expression of adhesion molecules in human umbilical vein endothelial cells. Journal of agricultural and food chemistry, 55(23), 9457-9464.

Han, S. M., Lee, K. G., Yeo, J. H., Kweon, H. Y., Woo, S. O., Lee, M. L., Park, K. K. (2007). Effect of honey bee venom on microglial cells nitric oxide and tumor necrosis factor-α production stimulated by LPS. Journal of Ethnopharmacology, 111(1), 176–181. https://doi.org/10.1016/j.jep.2006.11.008

Han, S. M., Lee, K. G., Yeo, J. H., Kweon, H. Y., Woo, S. O., Lee, M. L., Park, K. K. (2007). Effect of honey bee venom on microglial cells nitric oxide and tumor necrosis factor-α production stimulated by LPS. Journal of Ethnopharmacology, 111(1), 176–181.

Han, S. M., Lee, K. G., Yeo, J. H., Kweon, H. Y., Woo, S. O., Lee, M. Y., Park, K. K. (2007). Inhibitory effect of bee venom against ultraviolet B induced MMP-11 and MMP-3 in human dermal fibroblasts. Journal of Apicultural Research, 46(2), 94–98.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. cell, 144(5), 646-674.

Hanahan, D., Christofori, G., Naik, P., Arbeit, J. (1996). Transgenic mouse models of tumour angiogenesis: the angiogenic switch, its molecular controls, and prospects for preclinical therapeutic models. Eur J Cancer 32A, 2386– 93.

Hao, S., Yan, Y., Li, S., Zhao, L., Zhang, C., Liu, L., & Wang, C. (2018). The in vitro anti-tumor activity of phycocyanin against non-small cell lung cancer cells. Marine Drugs, 16(6), 1–15. https://doi.org/10.3390/md16060178

Heinen, T. E., & Gorini da Veiga, A. B. (2011). Arthropod venoms and cancer. Toxicon, 57(4), 497–511. https://doi.org/10.1016/j.toxicon.2011.01.002

Huh, J. E., Baek, Y. H., Lee, M. H., Choi, D. Y., Park, D. S., & Lee, J. D. (2010). Bee venom inhibits tumor angiogenesis and metastasis by inhibiting tyrosine phosphorylation of VEGFR-2 in LLC-tumor-bearing mice. Cancer letters, 292(1), 98-110.

Huh, J. E., Kang, J. W., Nam, D., Baek, Y. H., Choi, D. Y., Park, D. S., & Lee, J. D. (2012). Melittin suppresses VEGF-A-induced tumor growth by blocking VEGFR-2 and the COX-2-mediated MAPK signaling pathway. Journal of Natural Products, 75(11), 1922–1929.

Huh, J., E., Kang, J., W., Nam, D., Baek, Y., H., Choi, D., Y., Park, D., S. (2012). Melittin suppresses VEGF-A-induced tumor growth by blocking VEGFR-2 and the COX-2-mediated MAPK signaling pathway. J Nat Prod. 75. 1922-9.

Ibrahim, A. S., Khaled, H. M., Mikhail, N. N., Baraka, H., & Kamel, H. (2014). Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. Journal of Cancer Epidemiology, 1–18.

Ingbar, D. H. (2015). Fishman's pulmonary diseases and disorders. Annals of the American Thoracic Society, 12(8), 1255-1256.

Ip, S., Hadar, N., Keefe, S., Parkin, C., Iovin, R., Balk, E. M., & Lau, J. (2012). A Web-based archive of systematic review data. Systematic reviews, 1(1), 15.

J. Larry Jameson, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, J. L. (2018). Harrison's Principles of Internal Medicine. JAMA: The Journal of the American Medical Association (Vol. 243). https://doi.org/10.1001/jama.1980.03300470050029

Jameson, J., L., Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, J. L. (2018). Harrison's Principles of Internal Medicine. JAMA: The Journal of the American Medical Association (243), 234-239.

Jo, J., Whitcomb, D. J., Olsen, K. M., Kerrigan, T. L., Lo, S. C., Bru-Mercier, G., & Cho, K. (2011). Aβ 1–42 inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β. Nature neuroscience, 14(5), 545.

Kant Upadhyay, R. (2019). Use of Animal Venom Peptides/Toxins in Cancer Therapeutics. Current Trends in Biomedical Engineering & Biosciences, 16(4). 20-28

Kauczor, H. U., Bonomo, L., Gaga, M., Nackaerts, K., Peled, N., Prokop, M., ... & European

Society of Radiology (ESR. (2015). ESR/ERS white paper on lung cancer screening. European radiology, 25(9), 2519-2531.

Kauczor, H. U., Bonomo, L., Gaga, M., Nackaerts, K., Peled, N., Prokop, M., ... & Sculier, J. P. (2015). Lung cancer screening white paper: a slippery step forward? European Respiratory Journal, 46(5), 1521-1522.

Kim, J., Y., Kim, K., H., Lee, W., R., An, H., J., Lee, S., J., Han, S., M., (2015). Apamin inhibits PDGF-BB-induced vascular smooth muscle cell proliferation and migration through suppressions of activated Akt and Erk signaling pathway. Vascul Pharmacol., 7, 8-14.

Kit, R. M. (2012). June 2012 RNeasy ® Mini Handbook QIAGEN Sample and Assay Technologies.

Klenk, K. C., Tenenholz, T. C., Matteson, D. R., Rogowski, R. S., Blaustein, M. P., & Weber, D. J. (2000). Structural and functional differences of two toxins from the scorpion Pandinus imperator. Proteins: Structure, Function and Genetics, 38(4), 441–449.

Leu, C. M., Chang, C., & Hu, C. P. (2000). Epidermal growth factor (EGF) suppresses staurosporine-induced apoptosis by inducing mcl-1 via the mitogen-activated protein kinase pathway. Oncogene, 19(13), 1665–1675.

Levra, M. G., & Novello, S. (2012). Crizotinib: hunting another piece of the lung cancer genome. Lung Cancer Management, 1(3), 157-160.

Levra, M. G., Novello, S., Scagliotti, G. V., Papotti, M., & Le Cesne, A. (2012). Primary pleuropulmonary sarcoma: a rare disease entity. Clinical lung cancer, 13(6), 399-407.

Levra, M., Novello, S., Scagliotti, G. V., Papotti, M., & Le Cesne, A. (2012). Primary pleuropulmonary sarcoma: A rare disease entity. Clinical Lung Cancer. https://doi.org/10.1016/j.cllc.2012.05.003

Li, B., Lyu, P., Xi, X., Ge, L., Mahadevappa, R., Shaw, C., & Kwok, H. F. (2018). Triggering of cancer cell cycle arrest by a novel scorpion venom-derived peptide—Gonearrestide. Journal of Cellular and Molecular Medicine, 22(9), 4460–4473.

Li, L., Huang, J., & Lin, Y. (2018). Snake venoms in cancer therapy: past, present and future. Toxins, 10(9), 346.

Lindsey, S., & Langhans, S. A. (2015). Epidermal growth factor signaling in transformed cells. International review of cell and molecular biology, 314, 1–41.

Liu S, Yu., M., He, Y., Xiao, L., Wang, F., Song, C. (2008). Melittin prevents liver cancer cell metastasis through inhibition of the Rac1-dependent pathway. Hepatology. 47. 1964-73.

Lyons, S. A., O'Neal, J., & Sontheimer, H. (2002). Chlorotoxin, a scorpion-derived peptide, specifically binds to gliomas and tumors of neuroectodermal origin. GLIA, 39(2), 162–173.

Ma, R., Mahadevappa, R., & Kwok, H. F. (2017). Venom-based peptide therapy: Insights into anti-cancer mechanism. Oncotarget, 8(59), 100-108.

Marí-Beffa, P., Catena, A., Valdés, B., Cullen, D., & Houghton, G. (2007). N400, the reference electrode, and the semantic activation in prime-task experiments: A reply to Dombrowski and Heil (2006). Brain research, 1147, 209-212.

Masters. JR, 2000: Animal cell culture: a practical approach, New York: Oxford University Press, 3-10.

Mercurio, A., M., Bachelder, R., E., Bates, R., C., Chung, J. (2004). Autocrine signaling in carcinoma: VEGF and the alpha6beta4 integrin. Semin Cancer Biol. 14. 115– 22.

Moga, M. A., Dimienescu, O. G., Arvătescu, C. A., Ifteni, P., & Pleş, L. (2018). Anticancer activity of toxins from bee and snake venom-an overview on ovarian cancer. Molecules, 23(3), 1–21.

Moon, D. O., Park, S. Y., Heo, M. S., Kim, K. C., Park, C., Ko, W. S., Kim, G. Y. (2006). Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt. International Immunopharmacology, 6(12), 1796–1807.

Nam, K.-W., Je, K.-H., Kang, S. K., Han, H. J., Mar, W., Lee, J. H., & Lee, H. J. (2008). Inhibition of COX-2 activity and proinflammatory cytokines (TNF-α and IL-1β) production by water-soluble sub-fractionated parts from bee (Apis mellifera) venom. Archives of Pharmacal Research, 26(5), 383–388.

Olamendi-Portugal, T., GÓMEZ-LAGUNAS, F., GURROLA, G. B., & POSSANI, L. D. (1996). A novel structural class of K+-channel blocking toxin from the scorpion Pandinus imperator. Biochemical journal, 315(3), 977-981.

Oršolić, N. (2012). Bee venom in cancer therapy. Cancer and Metastasis Reviews. https://doi.org/10.1007/s10555-011-9339-3

Ouchari, L., Boukeskasse, A., Bouizgarne, B., & Ouhdouch, Y. (2019). Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. Biology Open, 8(2), bio035410.

Ownby, C. L., Powell, J. R., Jiang, M. S., & Fletcher, J. E. (1997). Melittin and phospholipase A2 from bee (Apis mellifera) venom cause necrosis of murine skeletal muscle in vivo. Toxicon, 35(1), 67-80.

Pal, P., Roy, S., Chattopadhyay, S., & Pal, T. K. (2015). Medicinal value of animal venom for treatment of Cancer in Humans - A Review. World Scientific News, 22, 128–144.

Pendás, A. M., Santamaría, I., Alvarez, M. V., Pritchard, M., & López-Otín, C. (1996). Fine physical mapping of the human matrix metalloproteinase genes clustered on chromosome 11q22.3. Genomics, 37(2), 266–269.

Pettitt, S. J., Krastev, D. B., Brandsma, I., Dréan, A., Song, F., Aleksandrov, R., Lord, C. J. (1959). Supplementary Information.

Putz, T., Ramoner, R., Gander, H., Rahm, A., Bartsch, G., Bernardo, K., … Thurnher, M. (2007). Bee venom secretory phospholipase A2 and phosphatidylinositol-homologues cooperatively disrupt membrane integrity, abrogate signal transduction and inhibit proliferation of renal cancer cells. Cancer Immunology, Immunotherapy, 56(5), 627–640.

Qian, C., Y., Wang, K., L., Fang, F., F., Gu, W., Huang, F., Wang, F., Z. (2015). Triple-controlled oncolytic adenovirus expressing melittin to exert inhibitory efficacy on hepatocellular carcinoma. Int J Clin Exp Pathol. 8, 10403-11.

Rady, I., Siddiqui, I. A., Rady, M., & Mukhtar, H. (2017). Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. Cancer Letters, 402(608), 16–31.

Raghuraman, H., & Chattopadhyay, A. (2007). Melittin: a membrane-active peptide with diverse functions. Bioscience reports, 27(4-5), 189-223.

Romeiro, Marilia Farignoli, Souza, William Marciel de, Tolardo, Aline Lavado, Vieira, Luiz Carlos, Colombo, Tatiana Elias, Aquino, Victor Hugo, Nogueira, Maurício Lacerda, & Figueiredo, Luiz Tadeu Moraes. (2016). Evaluation and optimization of SYBR Green real-time reverse transcription polymerase chain reaction as a tool for diagnosis of the Flavivirus genus in Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 49(3), 279-285.

Rowe, M. (2004). Rattlesnake: Portrait of a Predator. Manny Rubio. The Quarterly Review of Biology, 74(4), 477–477.

Rutkowski, P., & Ługowska, I. (2014). Follow-up in soft tissue sarcomas. Memo - Magazine of European Medical Oncology, 7(2), 92–96.

Samel, M., Vija, H., Kurvet, I., Künnis-Beres, K., Trummal, K., Subbi, J., Siigur, J. (2013). Interactions of PLA2-s from Vipera lebetina, Vipera berus berus and Naja naja oxiana venom with platelets, bacterial and cancer cells. Toxins, 5(2), 203–223.

Seto, T., Higashiyama, M., Funai, H., Imamura, F., Uematsu, K., Seki, N., Eguchi, K., Yamanaka, T., Ichinose, Y. (2006). Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer. Lung Cancer. 53. 91– 6.

Shahbazzadeh, D., Srairi-Abid, N., Feng, W., Ram, N., Borchani, L., Ronjat, M., & El Ayeb, M. (2007). Hemicalcin, a new toxin from the Iranian scorpion Hemiscorpius lepturus which is active on ryanodine-sensitive Ca2+ channels. Biochemical journal, 404(1), 89-96.

Shanbhag, V. K. L. (2015). Applications of snake venoms in treatment of cancer. Asian Pacific Journal of Tropical Biomedicine, 5(4), 275–276. https://doi.org/10.1016/S2221-1691(15)30344-0

Shibuya, M. (2006). Vascular endothelial growth factor (VEGF)-receptor2: Its biological functions, major signaling pathway, and specific ligand VEGF-E. Endothelium: Journal of Endothelial Cell Research, 13(2), 63–69.

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer Facts & Figures, 2019. CA: A Cancer Journal for Clinicians, 69(1), 7–34.

Sisakht, M., Mashkani, B., Bazi, A., Ostadi, H., Zare, M., Avval, F. Z., … Soukhtanloo, M. (2017). Bee venom induces apoptosis and suppresses matrix metaloprotease-2 expression in human glioblastoma cells. Brazilian Journal of Pharmacognosy, 27(3), 324–328.

Slightly, F., & The, H. (1986). 1. Discovery, producing organism 23) Staurosporine was originally discovered in 1977 from culture of,

306–310.
Snedecor, G. W., & Cochran, W. G. (1982). Statistical methods. 2nd printing. Lowa State Univ., Press, Ames, USA, pp507.
Soliman, E. M., Mahdy, S. E., Mossad, W. G., Hassanin, A. I., & El-Sayed, E. I. (2013). Effect of different inactivators on the efficacy of Egyptian foot and mouth disease SAT2 vaccine. J. Anim. Sci. Adv, 3(8), 388-395.
Son, D. J., Lee, J. W., Lee, Y. H., Song, H. S., Lee, C. K., & Hong, J. T. (2007). Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacology & therapeutics, 115(2), 246-270.
Soroceanu, L., Manning, T. J., & Sontheimer, H. (1999). Modulation of glioma cell migration and invasion using Cl− and K+ ion channel blockers. Journal of Neuroscience, 19(14), 5942-5954.
Stewart, B., & Wild, C. (2014). World Cancer Report 2014. International Agency for Research on Cancer, 22(1), 3–4.
Tahir, H., Coleman, C., Sagi, J., Wani, A., & Daruwalla, V. (2015). High-grade primary myxoid lung sarcoma presenting as recurrent hemorrhagic pleural effusions in a young woman. SAGE Open Medical Case Reports, 3, 2050313X1562435.
Tamaoki, T. (1991). Use and Specificity of Staurosporine, UCN-O1, and Calphostin C as Protein Kinase Inhibitors. Methods in Enzymology, 201(C), 340–347.
Tan, N., & Fung, S. (2008). Snake Venom L-Amino Acid Oxidases and Their Potential Biomedical Applications. Malaysian Journal of Biochemistry and Molecular Biology, 16(1), 1–10. Retrieved from http://ejum.fsktm.um.edu.my/article/597.pdf
Thuret, G., Chiquet, C., Herrag, S., Dumollard, J. M., Boudard, D., Bednarz, J., Gain, P. (2003). Mechanisms of staurosporine induced apoptosis in a human corneal endothelial cell line. British Journal of Ophthalmology, 87(3), 346–352.
Tsao, A. S. (2007). Lung Carcinoma: Tumors of the Lungs. Merck Manual Professional Edition, Online Edition. Retrieved from http://www.merckmanuals.com/professional/pulmonary-disorders/tumors-of-the-lungs/lung-carcinoma
Tseng, Y. L., Peng, H. C., & Huang, T. F. (2004). Rhodostomin, a disintegrin, inhibits adhesion of neutrophils to fibrinogen and attenuates superoxide production. Journal of biomedical science, 11(5), 683-691.
V Dubovskii, P., G Konshina, A., & G Efremov, R. (2014). Cobra cardiotoxins: membrane interactions and pharmacological potential. Current medicinal chemistry, 21(3), 270-287.
Varanda, E. A., & Tavares, D. C. (2005). Radioprotection: Mechanisms and Radioprotective Agents Including Honeybee Venom. Journal of Venomous Animals and Toxins, 4(1), 5–21.
Varanda, E. A., Monti, R., & Tavares, D. C. (1999). Inhibitory effect of propolis and bee venom on the mutagenicity of some direct-and indirect-acting mutagens. Teratogenesis, carcinogenesis, and mutagenesis, 19(6), 403-413.
Wang, D., Hu, L., Su, M., Wang, J., Xu, T. (2015). Preparation and functional characterization of human vascular endothelial growth factor-melittin fusion protein with analysis of the antitumor activity in vitro and in vivo. Int J Oncol. 47. 1160-8.
Wang, L., Li, D., Fu, Z., Li, H., Jiang, W., & Li, D. (2007). Association of CTLA-4 gene polymorphisms with sporadic breast cancer in Chinese Han population. BMC cancer, 7(1), 173.
Yadav, S. S., Prasad, C. B., Prasad, S. B., Pandey, L. K., Singh, S., Pradhan, S., & Narayan, G. (2015). Anti-tumor activity of staurosporine in the tumor microenvironment of cervical cancer: An in vitro study. Life Sciences, 133, 21–28. https://doi.org/10.1016/j.lfs.2015.04.019
Yoon, J., Jeon, J. H., Lee, Y. W., Cho, C. K., Kwon, K. R., Shin, J. E., ... & Yoo, H. S. (2012). Sweet bee venom pharmacopuncture for chemotherapy-induced peripheral neuropathy. Journal of acupuncture and meridian studies, 5(4), 156-165.
Zaky, F., & Sharkawi, E. (2015). 2, 3, 6(8), 3224–3235. https://doi.org/10.13040/IJPSR.0975-8232.6(8).3224-35.
Zhang, W. D., Cui, Y. Z., Yao, C. F., Jia, Q., Song, S. Q., Wang, Z. X., & Dong, Q. (2005). Polypeptide extract from scorpion venom inhibits angiogenesis and angiogenesis-dependent tumor growth. Chinese Pharmacological Bulletin, 21(6), 708–711.
Zhang, X., & Zhang, P. (2016). Scorpion venoms in gastric cancer (Review), 3683–3686.