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EFFECT OF BEE AND SCORPION VENOMS ON PROSTATE CANCER IN VITRO STUDY

By
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Abstract

Prostate cancer (PCa) is the commonest diagnosed visceral malignancy among males worldwide. Recent studies have shown that bee venom target the cancer cells without effect on the normal cells by activating PC3 with oxidative substances against prostate cancer. The induction of the apoptotic cell death through several cancer cell death mechanisms, includes activation of up regulation of c-myc, and c-met genes and down regulation of Casp-7, that are important to induce anticancer. Scorpion venom is a potential bio-source and therapeutic tool to design potent drugs against variety of diseases. It has been used as medicinal and therapeutic tool since ancient times in China. Scorpion venom consists of neurotoxins, salts, low molecular weight peptides and different enzymes with high molecular activities. These activities make them novel therapeutic agents. The results revealed low cytotoxicity of bee venom and satusporin while scorpion venom showed the highest level of cytotoxicity. On the other hand, apoptosis was shown in all tested subjects at pre G1, while cell cycle arrest was different, which was at G1 by scorpion venom and at G2M by both bee venom and satusporin. Apoptosis was at late stage by scorpion venom and satusporin drug while at an early stage by bee venom as Casp-7, C-myc, C-met showed up regulation, the highest level shown in scorpion venom. This study explains that bee venom and scorpion have a potencial therapeutic effect on prostate cancer.

Key words: Prostate cancer, *In-vitro*, Vemons, Bee, Scorpion, Apoptosis

Introduction

Generally, prostate cancer is the commonest cancer in men (apart from common skin cancers). The risk of prostate cancer increases with age. It is uncommon in men younger than 50 (Elabbady *et al*, 2014), although the risk is higher for younger men with a strong family history of prostate cancer, breast cancer or ovarian cancer (Schroder *et al*, 2014). Bee venom inhibits proliferation of cancer cell and tumor growth that involve stimulating of the local cellular immune responses in lymph nodes (Oršolić, 2012). Scorpion venom has anti-proliferative, cytotoxic, apoptogenic and immunosuppressive effects. Scorpion venom can be used against a vast variety of cancers like, human neuroblastoma, leukemia, glioma, brain tumor, breast cancer, melanoma, prostate cancer, and lung adenocarcenomas. Scorpion venom is very effective for treatment of various types of cancers and contains such peptides that have shown therapeutic potential against various cancers types (Gomes *et al*, 2009).

The functional significance of nerves in PCa proved that nerve-cancer cell interactions are as critical for epithelial homeostasis and PCa cell energetic metabolism and so it was suggested that nerves exert trophic effects on prostate cancer cells by regulation of basic cellular processes such as gene expression, protein translation, and metabolism (Coarfa *et al*, 2018).

This study aimed to describe the anti-cancer properties of Bee and Scorpion venoms *in vitro* and the possible mechanisms for how to treat prostate cancer.

Material and Method

Cell culture and MTT staining, penicillin-streptomycin, 25% trypsin-EDTA, & propidium iodide DNA staining were purchased (Sigma, USA). Cell culture grade dimethylsulfoxide (DMSO), medium RPMI1640, human prostate cancer (PC3) cell line was kindly supplied from R&D Sector, The International Center for Advanced Research. Scorpion venom (*Leiurus* sp. venom), bee venom

and satusporin drug were provided from ANDI COe, VACSERA, Egypt). 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-1641 media to the needed concentrations (Gajski *et al,* 2014). Maintenance of cell line and culture conditions: PC3 (human prostate adenocarcinoma 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 37^{O}C, 95% humidified atmosphere with 5% CO_2. Monolayer cells were trypsinized once reached 80% confluency (Masters, 2000). Cell counting: accurate cell number in suspension was calculated using haemocytometer (Soliman *et al,* 2013). Briefly, double fold dilution of the original cell suspension was prepared by adding 0.5mL of undiluted cell suspension to 0.5mL of 0.4% trypan blue dye. The mixture was mixed well and immediately aspirated to fill the haemocytometer counting chambers. Cytotoxicity using MTT reagent assay: Cells were plated in the 96-well tissue culture plate with 100µL of RPMI medium. 200µL of the materials were diluted serially twice. The plates were then incubated with the various venom concentrations for 24hr at 37ºC. For MTT assay, 96-well plate was washed by PBS and 50µL MTT were added into each well, and then incubated for 3hr at 37ºC. 50µL of isopropanol was added and incubated for 5 minutes at 37ºC. Plates were read by ELISA reader at wavelength 570nm, and absorbance was correlated with the cell number (Houghton *et al,* 2007). Cytotoxic effects of tested venoms on the MCF-7 cell lines were expressed as the IC_{50} value (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells). Inhibitory concentration (IC_{50}) of compounds was assessed by Masterplex software (2010). 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 70% ethanol. While, vortexing, pellet was added drop-wise. The cells were fixed for 30min at 4^{o}C 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 sock of RNase were 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 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). Gene expression analysis after treatment with venoms and Tamoxifen: The expression of regulatory and apoptotic genes (caspase7,C-Met, and C-myc) were examined using real-time PCR in PC3 cell lines. Primer sequences of oligonucleotides used for real time-PCR shown (Tab. 1). 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 (Bio-Rad Laboratories, USA) by real-time quantitative-PCR of RNA templates. cDNA synthesis and PCR amplification were done simultaneously. Melting curve analysis of PCR products was performed by Step One TM Real Time Systems software V.2.2.2.

Primer sequences for genes:

Casp7	F 5'-AGTGACAGGTATGGGCGTTCG-3',
Casp7	R 5'-GCATCTATCCCCCCTAAAGTGG-3'.
C-Met	F 5'-TGGTGCAGAGGAGCAATGG-3'
C-Met	R 5'-CATTCTGGATGGGTGTTTCCG-3'
C.myc	F 5'-TGCTGCCAAGAGGGTCAAGT-3'
C.myc	R 5'-GTGTGTTCGCCTCTTGACATTC-3'
β-actin	F 5'-GTGACATCCACACCCAGAGG-3'
β-actin	R 5'-ACAGGATGTCAAAACTGCCC-3

Statistical analysis: Data were processed statistically according to Snedecor and Cochran (1982), where minimum, maximum, mean value, standard deviation, standard error, and range were presented. Comparison between groups for significance was done using *t.* test.

Results

The results are shown in tables (1, 2 & 3) and figures (1, 2, 3 & 4)

Table 1: Material cytotoxicity (IC_{50})

No.	Sample code	IC_{50} ug/ml
		PC3
1	Bee venom	10.84531 ±0.62
2	Scorpion venom	2.342878 ±0.07
3	Satuosporin	10.34739 ±0.44

Table 2: Cell Cycle Analysis

Sample data (conc.ug/ml)		Results				
No.	Sample code	%G0-G1	%S	%G2/M	%Pre-G1	Comment
1	Bee venom	31.59	41.85	26.56	16.33	PreG1apoptosis&Cell growth arrest@G2/M
2	Scorpion venom	64.52	34.24	1.24	34.71	PreG1apoptosis&Cell growth arrest@G1
3	Satuosporin	28.72	41.25	30.03	22.41	PreG1apoptosis&Cell growth arrest@G2/M
4	Cont.pc3	44.39	48.26	7.35	1.64	

Table 3: Apoptosis.

No.	conc.um	Total	Early	Late	Necrosis
1	Bee venom	16.33	7.12	6.88	2.33
2	Scorpion venom	34.71	7.68	22.75	4.28
3	Satuosporin	22.41	5.61	14.61	2.19
4	cont.pc3	1.64	0.82	0.31	0.51

Table 4: Genes of tested material

No.	Sample code	Fld		
		Casp7	c-myc	c-met
		4.219569	0.335979	0.089634
1	Bee venom/PC3	18.71503	0.242974	0.064419
2	Scorpion venom/PC3	5.024099	0.26348	0.057583
3	satuosporin/PC3	1	1	1
4	cont.PC3			

Discussion

Among all the families: Buthidae is considered as the most fatal, poisonous and medically important family (Michael and Victor, 2003). Scorpion venom is very effective for the treatment of various types of cancers and contains such peptides that have shown therapeutic potential against various types of cancers (Gomes *et al,* 2009). Prostate cancer is the major cause of men death. It has been showed that polypeptide extract from scorpion venom PESV is potent against androgen independent-prostate cancer cell lines (Zhang *et al,* 2009). Also, the effects of bee

venom induce poison of apoptosis, necrosis, cytotoxicity, and growth inhibition on cancer cells. Bee venom inhibits proliferation of cancer cell and tumor growth that involve stimulating of the local cellular immune responses in lymph nodes (Oršolić, 2012). The sPLA2 enzymes can stimulate immunogenicity and tumor cell proliferation by various mechanisms. The main activity of sPLA2 is to catalytically digest cell membrane components and thus disrupts the integrity of the lipid bilayers making cells liable to more degradation. Direct protein interaction of PLA2 enzymes with cell surface receptors regulates a range of biological activities including proliferation (Mahady *et al,* 1998). PLA2 and melittin act synergistically, breaking up membranes of susceptible cells and enhancing their cytotoxic effect. The melittin induces membrane disruption via pores formation and membrane perturbation. Besides, cell death is induced by the activation of phospholipase A2 (PLA2) by melittin. This might be the main reason of the cytotoxicity of mellitin against several types of cancer cell. PLA2 plays a major role in the melittin-mediated membrane disruption via uncharacterized signal transduction mechanism (Dove *et al,* 2012). This study described the anticancer property of bee and scorpion venoms to determine their action mechanisms to treat prostate cancer cell line. This was done by evaluating the anticancer potentials through the cytotoxicity effect of venoms which measuring the cytotoxicity (IC_{50}), cell cycle arrest, apoptosis as well as pro-apoptotic and anti-apoptotic genes in treated and untreated cells compared the satusporine effect as synthetic anticancer drug.

Chaisakul *et al.* (2016) reported that animal venoms were neutralized during attacking cancer cells without impairing normal cells and tissues. But, these cytotoxic components can also increase the patient quality life from severe side effects. Hormone refractory prostate cancer (HRPC) remained a challenge but the finding of new promising agent is important against androgen independent prostate cancer (Kan *et al,* 2007). In the present study, evaluation of cytotoxicity on PC3 cells treated with scorpion venom (*Leiurus* sp.) for 24 hr, was 10.8 µg/mL disagree with Gomes *et al.* (2010) and El Fiky *et al.* (2018) who evaluated *Leurus* venom cytotoxicity on MCF-7 cells for 24, & 48 hrs. This difference may be due to change in the cell line. Depending on these results, that *Leiurus* venom is more toxic and potent than bee venom and satusporin standard anticancer drug. These results agreed with Omran (2003). On the other hand *Leiurus* venom has induced morphological changes and resulted in apoptotic cells include condensation and compartmentalization of nuclear and cytoplasmic materials (Omran, 2003). Moreover, several studies have demonstrated that scorpion venom can inhibit the growth and proliferation in cancer cell types (Aarti and Khusro, 2013; Calderon *et al,* 2014). Evaluation of cytotoxicity on PC3 cells treated with bee venom for 24 hr, was 2.3 µg/mL. these results was nearly agree with (Mohamed *et al,* 2016) who found that cytotoxicity was 3.7µg/mL in case of, MCF7 cell line in spite of difference in cell line, and disagree with IP *et al.* (2008) and El Fiky *et al.* (2018) that they found different values of bee venom cytotoxicity due to the use of different types of cell lines as they evaluate it on hep G2, A459, and MCF7 consequently. Concerning Cell cycle, treatment of PC3 with scorpion venom resulted in cell cycle arrest at G1 cell during incubation entering the subsequent stage and interrupted at this point. As a result, the antiproliferative activity of the venom was detected which block cell growth in G1/S and then induced apoptosis during Pre-G1. The result of this study supports the previous studies in-spite of the difference in cell line used. Gomes *et al,* (2010) reported that charybdotoxin (CTX) from *Leiurus* quinquestriatus venom was carried out a slight depolarization in breast cancer cells and subsequently arrested the cell cycle at the early G1, late G1, and S phases then gathered

cells in the S phase through induced the blockage of Ca^{2+}activated K^{+} channel. Chaubey (2017) reported that the blockage of specific ion channels may impair cancer growth and metastasis on cancerous cells and activate intracellular pathways which leading to cell cycle arrest and apoptosis. Also Cell cycle arrest of PC3 cells for 24 h treated bee venom, and satusporin were observed at G2M cell during incubation entering the subsequent stage and interrupted at this point. As a result, the anti-proliferative activity of the venom was detected which block cell growth in G1/S and then induced apoptosis during Pre-G1, this result of bee venom agreed with El Fiky *et al*, (2018) who gave same result in spite of difference in cell lines. Concerning apoptosis; PC3 cell line treated with both tested venoms after 24 h incubation, there was apoptosis in total, early, and late apoptosis as well as necrosis, but,there were some difference as it was in early stage in case of bee venom, while it was in late in case of the scorpion and satusporin. Concerning scorpion venom, this result disagree with (Omran, 2003) who reported that the rapid cytotoxicity effects of *leiurus* venom have been observed within 30 minutes and resulted in the lysis of the plasmalemma and organelle membranes leading to immediately cause the greatest level of cell population death. The effect of death in the breast T47D cancer cell line treated with *Leiurus* venom was observed by significant higher rates of dead cells in different concentration, this difference may be due to difference in cell line used. This was explained by Kastin (2013) who reported that when chlorotoxin binds with Cl^{-} channel in membrane protein, chlorotoxin interacts and kills the cancer cells through ion modulation. Besides, chlorotoxin can bind with MMP-2 resulted in reducing the expression of MMP-2 and inducing the regression of aggressive metastatic cancer cells. Also disagree with (Omran, 2003), who said that *Leiurus* venom induced an early significant apoptotic effect rather than necrosis on cancer cells. Furthermore, the apoptotic mediated by toxicity have effected on cell damage such as swelling, rupture, and necrosis. Also a polypeptide extract from scorpion venom (PESV) has been isolated from *Buthus martensi* Karsch (Bmk). PEVS is a peptide with 50-60 amino acids and has antiproliferative, cytotoxic and apoptosis-induced activities against Human Umbilical Vein endothelial Cell (HUVEC), inhibition of neovascularization, suppression of tumor growth of S180 sarcoma and H22 hepatocelluar carcinoma in mice (Zhan *et al,* 2005). Also, these results may be due to the effect of Scorpion venoms on targeting cancerous cell causing proliferation, migration, invasion, neovascularization and apoptotic activity (Chaisakul *et al,* 2016). As a result, these cytotoxins can potentially induce apoptosis by forming apoptotic body, increasing in sub G1 population, fragmenting DNA of the cells and cleaving the poly (ADP-ribose) polymerase (PARP) (Gomes *et al,* 2010). Based on the activity of Na^{+}, K^{+}, Ca^{++} and Cl^{-} ion channels have the ability to alter the abnormal expression binding to cancer processes involving cell volume and motility as well as cell proliferation and death (Ding *et al,* 2014). Concerning bee venom our result revealed that apoptosis by bee venom was in early stage, this result was nearly agree with (El Fiky *et al,* 2018) inspite of difference in cell line used. This result may be due to activation of intrinsic and extrinsic pathways (Erdeş *et al,* 2014). Bee venom affected death receptors (DR) leading to apoptosis through the interaction with TNF cytokine family such as tumor necrosis factor (TNF) with death receptor (DR1), Fas ligand (FASL) with death receptor 1(DR2) and Apo3 ligand (Apo3L) with death receptor 3(DR3), (El Fiky *et al,* 2018). Satusporin and its analogues have the ability to induce the death of human breast cancer cells among both in vivo and in vitro studies. In addition, its metabolites can cause morphological changes by the cleavage of DNA leading to induce apoptosis on cells (Wilson

et al, 1995).

Concerning Genes (pro- and anti-apoptotic genes): This study observed the gene expression level of pro-apoptotic gene as Caspase-7 and anti-apoptotic genes as c-myc, and c-met on PC3 cell line after the treatment with the IC_{50} of *Leiurus* venom using real-time PCR. Subsequently, it was found that there was increase in expression of caspase-7 under the effect of scorpion venom than bee venom and Satusporin. Concerning scorpion venom this study is in agreement with results evaluate the up regulation of Capsase-3 gene in cancer cell lines treated with scorpion venom leading to induce apoptosis, as both caspase-3 and caspase-7 are pro-apoptotic genes. Ma *et al.* (2017) reported that ADAM proteins and matrix metalloproteinases can active HER2 receptor targeted for cancer cells as proteolytic enzymes from both venoms carried out their mechanism of action through proteolytic cleavage of growth factors and corresponding receptor (HER2) which are causing the degradation of extracellular matrix and inhibiting growth signaling. Also Scorpion venom induces anti-proliferative effect by arresting S-phase and causing apoptosis through Capase-3 activity, nitric oxide production, DNA fragmentation and depolarization of mitochondrial membrane (Ahluwalia and Shah, 2014). The expression level of anti-apoptotic gene (C-myc, and C-met) on PC3 cell line after the treatment with the IC_{50} of *Leiurus* venom using real-time PCR, there was down regulation of genes after the treatment in a high significance way, the results demonstrate the down regulation of both genes in prostate cancer cell lines treated with scorpion venom. Erdes *et al.* (2014), reported that *Leiurus* venom has a proliferative effect on adenocarcenoma cell line by venom components. *Leiurus* quinquestriatus venom has anticancer effect on MCF-7 cell line (Gomes *et al,* 2010). Moreover, *Leiurus* quinquestriatus venom has also exhibited a broad range of anticancer activity on MDA MB 231 (ER-) cell line (Al-Asmari *et al,* 2015). Scorpion venom decreases Stat3 on cancer lines, thereby decreases the expression of antiapoptotic gene (Al-Asmari et al., 2017). Concerning bee venom it showed up regulation in casp-7 and upregulation with c-myc, and c-mat. These result agreed with Baud and Karin (2001) and Choi *et al.* (2014). These result may be owed to TNFR1 induces receptor trimerization and recruitment of TNFR1-associated death domain protein (TRADD), which is responsible for recruiting receptor-interacting protein 1 (RIP1), Fas-associated death domain protein (FADD), TNF-receptor-associated factor 2 (TRAF2). FADD and RIPK recruitment by TRADD results in caspase cascade activation that leads to induction of apoptosis via activation of initiator caspases caspase-2, -8, & -10 by the activated receptor TNFR1. Initiator caspases cleave and activate effector caspases-3, -6 and -7 (Baud and Karin, 2001). Fas bind to Fas ligands (FasL) and FADD forming death-inducing signaling complex (DISC). DISC is responsible for recruiting initiator caspases as caspase 8 which in turn cleaves and activate caspase 3 and 7 to induce cell apoptosis (Choi *et al,* 2014). Apo-3 ligand will bind to the death receptor 3 resulting in induction of FADD which in turn recruit procaspase 8. Procaspase 8 is then activated via autocatalysis resulting in active caspase 8. Apoptosis is stimulated via activated caspase 8 through two different cascades. The first cascade is induction of apoptosis directly via cleavage and activation of caspase 3 by caspase 8 (Choi *et al*, 2014). Also, through the extrinsic apoptotic signaling, bee venom induced apoptosis via Fas receptor. This results in the release of cytosolic proteins as Bcl-2. These proteins will target the mitochondria and change its membrane potential causing it to swell and make it leaky. This would lead to release of the apoptotic effector proteins in the cytosol including the secondary mitochondria derived activator of caspase (SMAC) and cytochrome C (IP *et al,* 2008). The cytochrome C would bind to Apaf-1

and procaspase-9 forming apoptosome that was the final irreversible stage of apoptosis. The initiator caspase-9 will activate the effector caspase-3 and induce apoptosis of the cells via damaging mutated DNA (IP *et al*, 2008). The bee venom inhibited cancer cell growth by induction of apoptotic cell death in PC-3 human prostate cancer cells. The effects were mediated by the suppression of constitutively activated NF-κB. Bee venom and melittin decreased anti-apoptotic proteins but induced proapoptotic proteins. However, pan caspase inhibitor abolished bee venom and melittin-induced apoptotic cell death and NF-κB inactivation. Bee venom administration to nude mice implanted with PC-3 cells resulted in inhibition of tumor growth and activity of NF-κB accompanied with apoptotic cell death. Therefore, these results indicated that bee venom and melittin could inhibit prostate cancer in in vitro and in vivo, and these effects may be related to NF-κB/caspase signal mediated induction of apoptotic cell death. On the other hand, the satusporin cause activation of Caspase-7, these result in agreement with (Moriai *et al*, 2008) who reported that Caspase-3, Caspase-7, Caspase-8, & Caspase-9 was observed to be activated after the treatment of breast cancer cells with satusporin. It can induce the potential activity of pro apoptotic genes as Caspase-3, & Caspase-7 in other type of adenocarcenoma as ER-negative breast cancer cell mediating by cleavage of the fluorogenic tetrapeptide substrate and the poly (ADP-ribose) polymerase (Mandlekar *et al*, 2000).

Conclusion

The study reinforced the idea after bee and more potently scorpion venoms that they can act as anti-cancer agents when compared their effects to satusporine the anticancer drug and specifically prostate cancer cell line since it is the commonest cancer and causing death among males and hence of great importance. It compared the cytotoxic effect, gene expression effects and selective apoptopsis to cancer cells of prostate cell line experimentally with results encouraging to use both bee and scorpion venoms as potential biological agents for various types of cancers on the forth coming future. Data were comparable to other previous cancer cell lines effects done also experimentally before.

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References

Baud, V, Karin, M, 2001: Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol. 11, 9:372-7

Chaisakul, J, Hodgson, WC, Kuruppu, S, Prasongsook, N, 2016: Effects of animal venoms and toxins on hallmarks of cancer. J. Cancer 7, 11:1571-8.

Chaubey, KM, 2017: Scorpion venom**:** Pharmacological analysis and its application., Euro J. Biol. Res. 7, 4:271-90.

Choi, KE, Hwang, CJ, Gu, SM, Park, M H, Kim, JH, *et al*, 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 (Basel). 6, 8:2210-28.

Coarfa, C, Florentin, D, Putluri, N, Ding, Y, Aum J, *et al*, 2018: Influence of the neural microenvironment on prostate cancer. Prostate 78, 2:128-39.

Ding, J, Chua, P, Bay, B, Gopalakrishnakone, P, 2014: Scorpion venoms as a potential source of novel cancer therapeutic compounds, Exp. Biol. Med. (Maywood). 239, 4:387-93.

Dove, J, Keith, A, Eshleman, J, Janowsky, A, 2012: Melitin stimulates fatty acid release via non-phospholipase-mediated mechanisms and interacts with the dopamine transporter and other membrane spanning proteins. Eur. J. Pharmacol. 650, 2.3:501-10.

El Fiky, AA, Mohsen, R, Mohamed, AF, Wahba, EM, 2018: Experimental evaluation of doxorubicin drug as anticancer agent using anural component an in vitro study. J. Egypt. Soc. Parasitol. 48, 3: 699- 712

Elabbady A, Eid, A, Fahmy, A, Kotb AF, 2014: Pattern of prostate cancer presentation

among the Egyptian population: A study in a single tertiary care center. Cent. Euro. J. Urol. 67:351-6.

Erdeş, E, Doğan, TS, Coşar, İ., Danışman, T, Kunt, KB, *et al*, 2014: Characterization of Leiurusabdullahbayrami (Scorpiones: Buthidae) venom: peptide profile, cytotoxicity and antimicrobial activity. J. Venom. Anim. Toxins Inclu. Trop. Dis. 20, 1:48.

Gomes, A, Bhattacharjee, P, Mishra, R, Biswas, KS, Dasgupta, C, *et al*, 2010: Anticancer potential of animal venoms and toxins. Indian J. Exp. Biol. 48:93-103.

Gomes, A, Haldar, S, Giri, B, 2009: Experimental osteoporosis induced in female albino rats and its antagonism by Indian black scorpion (*Heterometrus bengalensis* C.L.Koch) venom. Toxicon 53:60-8.

Ip, S, Liao, S, Lin, S, Lin, J, Yang, J, *et al*, 2008: The role of mitochondria in bee venom-induced apoptosis in human breast cancer MCF7 cells. In vivo 22, 2:237-45.

Kan, SF, Yu, CH, Pu, HF, Hsu, JM, Chen, M J, *et al*, 2007: Anti-proliferative effects of evodiamine on human prostate cancer cell lines DU145 and PC3. Cell Biochem. 101:44-56.

Kastin, AJ, 2013: Handbook of Biologically Active Peptides Book. 2nd Ed. ISBN 978-0-12-3850959

Mahady, GB, Liu, C, Beecher, CW, 1998: Involvement of protein kinase and G proteins in the signal transduction of benzophenanthridine alkaloid biosynthesis. Phytochemistry 48, 1:93-102.

Mandlekar, S, Hebbar, V, Christo, K, Kong, AN, 2000: Pharmacodynamics of tamoxifen and its 4-hydroxy and N-desmethyl metabolites: activation of caspases and induction of apoptosis in rat mammary tumors and in human breast cancer cell lines. Exp. Biol. Med. (Maywood). 239, 4:387-93.

Michael, ES, Victor, F, 2003: High-level systematics and phylogeny of the extant scorpions (Scorpiones: Orthosterni). Euscorpius Occas. Public. Scorpiol.11:1-175

Mohamed, AF, El Fiky AA, Hassan, OS, El-Megharbei, NA, 2016: Controlled study of application of bee product as new medicine against breast cancer. Int. J. Adv. Res. 4, 6:392-402 ·

Omran, MAA, 2003: In vitro anticancer effect of scorpion *Leiurus quinquestriatus* and Egyptian Cobra venom on human breast and prostate cancer cell lines. J. Med. Sci. 3, 1: 66-86.

Oršolić, N, 2012: Bee venom in cancer therapy cancer Metast. Rev. 31, 1/2:173-94

Oršolić, N, Knežević, A, Šver, L, Terzić, S, Hackenberger, BK, *et al*, 2012: Influence of honey bee products on transplantable murine tumors. Vet. Comp. Oncol. 1, 4:216-26.

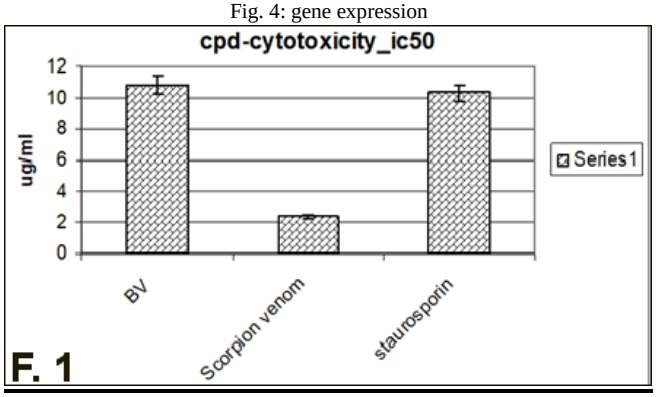
Schroder, FH, Hugosson, J, Roobol, MJ, Tammela, TL, Zappa, M, *et al*, 2014: Screening and prostate cancer mortality: results of the European randomized study of screening for prostate cancer (ERSPC) at 13 years of follow up. Lancet 384, 9959:2027-35.

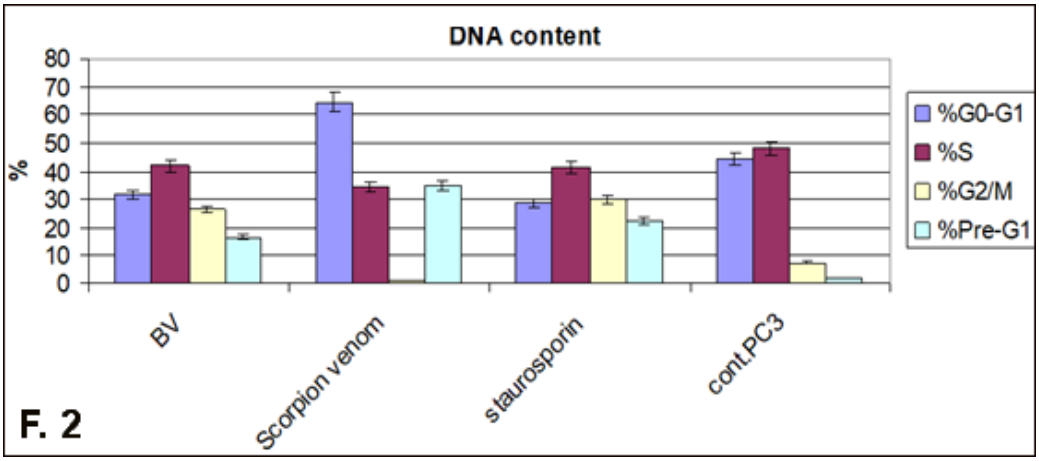
Wilson, WJ, Wakeling, EA, Morris, DI., Hickman, AJ, Dive, C, 1995: MCF-7 human mammary adenocarcinoma cell death in vitro in response to hormone-withdrawal and DNA damage. Int. J. Cancer 61:502-8.

Zhang, WD, Cui, YZ, Yao, CF, Jia, Q, Song, SQ, *et al*, 2005: Polypeptide extract from scorpion venom inhibits angiogenesis and angiogenesis-dependent tumor growth. China Pharmacol. Bull. 22:708-11

Explanation of figures

Fig. 1: Cytotoxicity ic50
Fig 2: DNA content
Fig. 3: Apoptosis
Fig. 4: gene expression




DNA content
%
80
70
60
50
40
30
20
10
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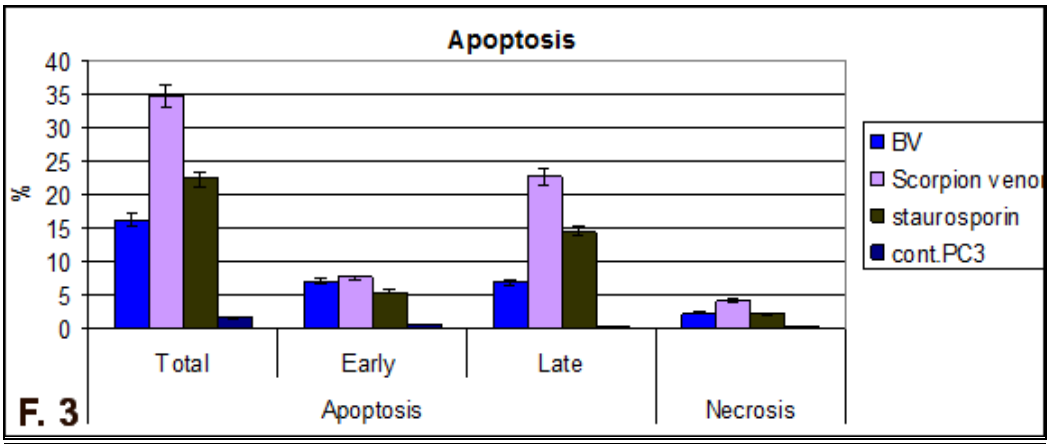
BV
Scorpion venom
staurosporin
cont.PC3
%G0-G1
%S
%G2/M
%Pre-G1

F. 2


Apoptosis
%
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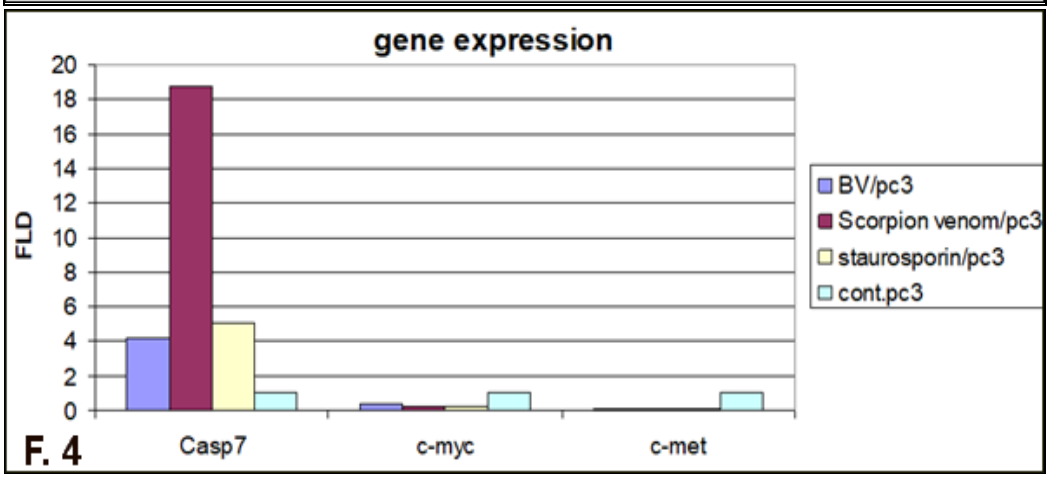
Total
Early
Late
Apoptosis
Necrosis
BV
Scorpion veno
staurosporin
cont.PC3

F. 3


gene expression
FLD
20
18
16
14
12
10
8
6
4
2
0
Casp7
c-myc
c-met
BV/pc3
Scorpion venom/pc3
staurosporin/pc3
cont.pc3

F. 4