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RESEARCH ARTICLE

EVALUATION OF ANTICANCER ACTIVITY OF SOME VENOMOUS ANIMAL TOXINS ON HUMAN BREAST AND COLON CANCER CELL LINES AND RELATED ANTIOXIDANT PROFILE

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Abstract

Background:- Breast and colon cancers are the most types of cancer in Egyptian patients. Radiation and chemotherapy have undesirable side effects, so the drugs are used instead. Venoms have been used as folk medicine since ancient times.

Purpose and aim:- This study examines the anticancer potential of Egyptian snake (*Naja haje*), scorpion (*Leiurus quinquestriatus*) and bee (*Apis mellifera*) venoms then compared to a synthetic anticancer drug 5-Flurouracil (5-FU).

Methods:- The cytotoxicity activity of tested venoms was determined using SRB assay. Antioxidant potential was determined by the evaluation of the variation in MDA, NO. and GSH content. RNA was extracted to evaluate the expression of P53, Bcl2 and Bax genes using semi-quantitative RT-PCR analysis after 24hrs. treatment.

Results & Discussion:- MCF-7 cells were more sensitive than Caco-2 cells to tested venoms while 5-FU was more toxic to both cell lines. The *Naja h.*, *Leiurus q.* and *Apis m.* venoms had IC_{50} as 3.5, 27.8 and 2.5 µg/ml with MCF-7 cell line and 20, 32.3 and 14 µg/ml with Caco-2 cell line respectively. The *Naja h.* venom had apoptotic effect against MCF-7 and Caco-2 cell lines through the elevation of p53 and depletion of Bcl-2 gene expression with increasing NO. and decreasing in GSH contents. The *Leiurus q.* venom had apoptotic effect against MCF-7 and Caco-2 cell lines through the elevation of Bax and depletion of Bcl-2 gene expression with elevation in NO. level. The *Apis m.* venom had apoptotic effect against MCF-7 and Caco-2 cell lines through the elevation of p53 and Bax with depletion of Bcl-2 gene expression and increasing NO. and decreasing in GSH contents. While 5-FU was toxic through depletion of Bcl-2 gene expression and increasing NO. level.

Conclusion:- Finally, it can be concluded that snake (*Naja h.*), scorpion (*Leiurus q.*) and bee (*Apis m.*) venoms had anticancer

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potentials on human breast and colon cancer cells and this is positively related to the antioxidant profile and apoptotic gene expression.

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Introduction:-

Cancer has characterized features as an accelerated cell proliferation and uncontrolled growth of a set of abnormal cells which has insufficient apoptosis and metastasis ability **(Jain, 2014)**. Breast cancer is the second most common cancer worldwide and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases (11% of all cancers) followed by colon cancer incidence was estimated 1.4 million (9.7% of all cancers) diagnosed in 2012 **(Ferlay *et al*., 2015).**

The use of traditional drugs is declining and naturally extracted drugs are used instead. The bioactive molecules in animal venoms have stimulated new pharmaceutical discoveries **(Harvey, 2014).**Several pharmacological anticancer applications have been found in many venomous animals, such as snakes, scorpions, bees, spiders and frogs **(Gomes *et al*., 2010).** Captopril and Escozul medicines are derived from snake and scorpion venoms are used to treat cancers **(Bryan, 2009; Lorenzo *et al*., 2012)**.

The medical value of venoms is important because of its complex bioactive components that are characterized by its high degree of specificity. Up till now, a number of studies have investigated the effects of snake, scorpion and bee venoms as an effective tool in cancer therapy development **(Vyas *et al*., 2013; Ortiz *et al*., 2015; Zheng *et al*., 2015).**

Snake venom is a complex mixture of bioactivity peptides and proteins that have cytotoxic, antitumor and apoptotic effect on different cancer cells **(Corrêa *et al*., 2002; Son *et al*., 2007; Song *et al*., 2012; Badr *et al*., 2014).** Collectively, these studies outline a critical role in biotherapy of cancer for these components of snake venom like Atroporin from *Crotalus atrox*, Kaotree from *Naja naja kaouthia*, Trigramin from *Trimeresurus gramineus*, Rhodostomin from *Calloselasma rhodostoma* and Contortrostatin from *Agkistrodon contortrix* **(Swaim *et al*., 1996; Lipps, 1999; Zhou *et al*., 2000; Yeh *et al*., 2001).**

Scorpion venom is a complex mixture of salts, nucleotides, biogenic amines, enzymes, mucoproteins, as well as peptides and proteins. Due to the medical relevance of scorpion venoms have been applied in traditional medicine, mainly in Asia and Africa for thousands of years **(Goudet *et al*., 2002; Shao *et al*., 2007).** Thus far, some studies have focused on some components of scorpion venom that have an antitumor effect like Bengaline from *Heterometrus bengalensis*, Iberiotoxi from *Buthus tamulus*, Margatoxin from *Centruroides margaritatus*, Chlorotoxin from *Leiurus quinquestriatus* and Neopladine 1 and 2 from *Tityus discrepans* **(Galvez *et al*., 1990; Debin *et al*., 1993; Garcia-Calvo *et al*., 1993; D'Suze *et al*., 2010; Gupta *et al*., 2010).**

The medical use of bee venom has been known since ancient times due to its healing properties. It can be seen in many religious texts including the Quran, Bible and Veda. The first injectable form of bee venom is developed by Dr. Franz Kretsky (1928) from Austria**(Kim, 2013)**. Mellitin and apamin are the most important peptides in bee venom that have an antitumor effect **(Zhou *et al*., 2013).**

P53, BCL-2and BAX are proteins play important role in the control of apoptosis. P53 is a protein which plays a critical role in the intrinsic apoptosis pathway activation. Bcl-2 may act upstream of the p53 pathway. The induction of Bax mRNA and protein were found to be stuck in the presence of Bcl-2. The cross-like between Bcl-2, p53 and Bax genes is a significant determinant of drug-induced apoptosis **(Fesik, 2005; Giménez-Bonafé *et al*., 2009).**

An important therapeutic strategy was suggested to use antioxidant inhibitors and/or reactive oxygen species (ROS)-generating compounds to generate apoptosis in cancer cells **(Haddad, 2004).** ROS stimulate cell death by inducing apoptosis through provide lipid peroxidation and DNA damage. The end product of lipid peroxidation reacts with deoxyadenosine and deoxyguanosine in DNA leading to DNA fragmentation **(Marnett, 1999; Barrera *et al*., 2008).** GSH depletion affects apoptosis sensitivity by the activation of death signals (caspases 3,8 and 9) and leads to overexpression of Bax and P53 and inhibition of Bcl-2 expression **(Higuchi, 2004; Franco and Cidlowski,**

2009). High concentration of NO. induces cell apoptosis while lower concentration can be antiapoptotic **(Nicotera *et al.,* 1995; Dimmeler *et al.,* 1997).**

So this work aims to evaluate the possible anticancer effects of some natural venoms of *Naja h. Leiurus q*. and *Apis m.,* compared to a synthetic anticancer drug (5-FU). The evaluation parameters include cell viability, the expression of some genes related to apoptosis by semi-quantitative RT-PCR technique and oxidant/antioxidant system analysis.

Material and Methods:-

5-Fluorouracil, 250mg/ 5mL from EBEWE Pharma Company was diluted in RPMI-1641 media (BioWhittaker™ Classical Media, Lonza) to prepare test concentrations.

Preparation of venoms:-

Dried snake venom of Egyptian copra *Naja haje* and scorpion venom of the deathstalker *Leiurus quinquestriatus* were kindly supplied from Sera plant VACSERA, Dokky, Giza, Egypt and bee venom of Egyptian bee *Apis mellifera* was supplied from Economic Entomology and Pesticides Department of Faculty of Agriculture Cairo University, Giza, Egypt. Both were dissolved in 2.5mL sterile double distilled water to contain 10mg/mL and sterilized using 0.22 m syringe filter (Millipore-USA) and serially diluted in RPMI-1641 media (BioWhittaker™ Classical Media, Lonza) to the needed concentrations **(Gajski *et al.,* 2014).**

Cell lines and culture conditions:-

MCF-7 (Human breast cancer cell line, HTB-22) and Caco-2 (Human colon cancer cell line, HTB-37) and trypsin 0.25% were provided by VACSERA - Cell Culture Unit, Dokky, Giza, Egypt. These cell lines were originally supplied from the American Type Culture Collection (ATCC). Cells were seeded at a density of 2×10^5/ml and routinely cultured in RPMI-1641 medium in tissue culture flasks (Griener, Germany). This medium was supplemented with 5% fetal bovine serum plus 1% penicillin-streptomycin and 1% non-essential amino acids. Then the flasks were incubated at 37^{o}C in a humidified chamber and 5% CO_2 atmospheric condition. When the cells reached confluent monolayer, the cells were trypsinized according to **(Masters, 2000).**

Cytotoxicity using sulphodiamine-B assay (SRB):-

MCF-7 and Caco-2 cells were seeded at the density of 4×10^4/mL into 96-well cell culture plates (Nune, USA) in the growth medium. After the confluence of cells, they were treated with different concentrations of test venoms and 5-FU (0.01, 0.1, 1, 10 and 100 µg/ml) as a reference anticancer drug. At 48 h, cells were treated for 1 h with 100 µL of 10% TCA at 4°C and stained with 0.4% (W/V) SRB in 1% (V/V) acetic acid. The optical density was measured at 570 nm after reformation of the dye in 10 mM Tris base (Sigma-Aldrich, USA) using ELIZA reader (Dynatec medical products, England) according to **(Houghton *et al.,* 2007).** TCA**,** SRB and acetic acid were purchased from (Sigma-Aldrich, USA).

$$The\ Viability\ percentage\ \% = \frac{O.D.\ of\ treated\ cells}{O.D.\ of\ untreated\ cells} \times 100$$

The half-maximal growth inhibitory concentrations were calculated using (Sigma Plot software).

Morphological changes:-

Morphological changes were detected 24hrs.post MCF-7 and Caco-2 cells treatment with different test venoms and 5-FU IC_{50} values. Cells were investigated using inverted microscope (Leica, Germany) at 20 X magnification**.**

RT-PCR (Reverse Transcriptase Polymerase Chain Reaction):-

The primer sequences of the oligonucleotides used for PCR were (Table 1) as follows: GAPDH, sense: 5′ CAA GGT CAT CCA TGA CAA CTT TG 3′, antisense: 5′ GTC CAC CAC CCT GTT GCT GTA G 3′; P53, sense: 5′ TCA GAT CCT AGC GTC GAG CCC 3′, antisense: 5′ GGG TGT GGA ATC AAC CCA CAG 3′; BAX, sense: 5′ ATG GAC GGG TCC GGG GAG CA3′, antisense: 5′ CCC AGT TGA AGT TGC CGT CA3′; Bcl2, sense: 5′ GTG AAC TGG GGG AGG ATT GT 3′, antisense: 5′ GGA GAA ATC AAA CAG AGG CC 3′. Total RNA was extracted by using RNeasy® Mini Kit (QIAGEN®) according to manufacturer's instruction. Total RNA (2µg) was applied for the synthesis of cDNA with QuantiTect® Reverse Transcription Kit (QIAGEN®). PCR was performed in a DNA Thermal Cycler (Applied Biosystems) using QuantiTect SYBR Green PCR Master Mix. The melting curve analysis of the PCR products was performed by StepOneTM Real-Time Systems software V.2.2.2.

Biochemical evolution:-

Oxidative stress marker:-

The cells were treated with the IC_{50} concentration of venoms and 5-FU for 24h. After 24hrs. post treatment, the cell suspensions were centrifuged. The cell pellets were suspended with PBS (BioWhittaker™ Classical Media, Lonza) and moved into reaction tubes.

Nitric Oxide (NO.):-

NO. level was measured as total nitrite concentration with Griess method **(Green *et al.*, 1982)**. Griess reagents (1:1) of Sulfanilamide and NEDD were added one by

Table 1:- primers used in RT-PCR assay.

The gene	Primer sequences	Annealing temperature
GAPDH	**F: 5′ CAA GGT CAT CCA TGA CAA CTT TG 3′**	**48°C**
	R: 5′ GTC CAC CAC CCT GTT GCT GTA G 3′	**53°C**
P53	**F: 5′ TCA GAT CCT AGC GTC GAG CCC 3′**	**53°C**
	R: 5′ GGG TGT GGA ATC AAC CCA CAG 3′	**51°C**
Bax	**F: 5′ ATG GAC GGG TCC GGG GAG CA3′**	**55°C**
	R: 5′ CCC AGT TGA AGT TGC CGT CA3′	**49°C**
Bcl-2	**F: 5′ GTG AAC TGG GGG AGG ATT GT 3′**	**49°C**
	R: 5′ GGA GAA ATC AAA CAG AGG CC 3′	**47°C**

F: Forward strand and **R:** Reverse strand

one, mixed and left 10 min in dark at 25°C. The absorbance of the supernatant was measured photometrically at 540 nm.

Malondialdehyde (MDA):-

Lipid peroxidation was measured by the thiobarbituric acid assay **(Draper and Hadley, 1990)**, which measures the production of MDA. 10% trichloroacetic acid was added to cell suspension. The tubes were placed in a water bath and kept at 95 ◦C for 15 min. After cooling, the suspensions were centrifuged at 3000 rpm for 10 min. 0.67% thiobarbituric acid was added. The tubes were placed in a water bath and kept at 95 ◦C for 15 min. After cooling, the absorbance of the supernatant was measured photometrically at 532 nm.

Antioxidative enzymes (Reduced Glutathione GSH):-

The procedure to estimate the reduced glutathione (GSH) level was according to **(Ellman, 1959)**. The suspended cells were added with sulfuric acid and mixed well. Tubes were left for 10 min at 25°C. The addition of tungstate solution was followed by mixing for 5 min and centrifugation for 20 min at 860 rpm. After 30-60 seconds of the addition of Tris buffer and DNTB reagent to the clear supernatant, the solution colors were measured at 412 nm.

Statistical Analysis:-

For statistical comparison of results from different experiments, data were analyzed using one way ANOVA and T-test at significance level ($P < 0.05$) using Graphpad Prism 5 (Graphpad software, USA)

Results:-

Cytotoxicity by sulphodiamine-B assay (SRB):-

MCF-7 cells was treated with venoms derived from *Naja h.*, *Leiurus q*. and *Apis m.* and 5-FU as a standard anticancer drug using 0.01, 0.1, 1, 10 and 100 µg/ml for 48 hours compared with a control of untreated cells. Data recorded revealed that viability percentage and concentration were related. The cytotoxic effect 5-FU wasn't significantly related to the effect of *Leiurus q.* venom ($P > 0.05$). In the meantime, there was a significant correlation between the effect of 5-FU and the effect of *Apis m.* and *Naja h.* venoms ($P > 0.05$). Also, there was a non-significant difference in the cytotoxicity of venoms namely *Apis m.* and *Naja h*. (Table 2). Regarding Caco-2 cell, data revealed that the viability percentage was concentration dependent. There was a significant difference of venoms toxicity compared with in MCF-7 cell line due to the difference between effects of test venoms in both cell lines ($P > 0.05$). Except in case comparison between *Naja h.* and *Apis m.* venoms, the viability was the least in *Naja h.* venom compared with others ($P < 0.05$) (Table 3). The data recorded revealed the toxicity of test venoms showing a variable reactivity which was cell line and concentration dependent. The IC_{50} of *Apis m.* venom is non-

significantly toxic in compression with *Naja h*. venom (P>0.05) while both were significantly toxic compared to the *Leiurus q*. venom recording IC_{50} values of 2.5, 3.5 and 27.8 µg/ml for MCF-7 (Table -1). At the same time, the IC_{50} of test venoms to Caco-2 cells showed that there was a significant toxicity of *Naja h.* venom to both *Apis m.* and *Leiurus q*. venoms by 20, 32.3 and 14 µg/ml respectively. 5-FU as a positive control showed a significant toxicity to both cell-lines than test venoms recording IC_{50} values of 0.5 and 2.6 µg in case of MCF-7 and Caco-2 respectively (Table 4).

The IC_{50} values of 5-FU, *Naja h*., *Leiurus q*. and *Apis m.* venoms was summarized in (Table 3). Data recorded showed that 5-FU as chemotherapeutic agent has a highly cytotoxic effect on both MCF-7 and Caco-2 cells more than venoms as bio-therapeutic agents. *Naja h.*, *Leiurus q.* and *Apis m.* venoms had a medium, low and high cytotoxic effect on both MCF-7 and Caco-2 cells respectively.

Table 2:- Percentage viability of MCF-7 cells post 48 hrs. treatment *Naja h*. venom, *Leiurus q.* venom and *Apis m*. venom and 5-FU relative to concentration.

Concentration (µg/ml)	Percentage viability of MCF-7 cells treated with			
	Naja h.	***Leiurus q.***	***Apis m.***	**5-FU**
0	**100**	**100**	**100**	**100**
0.01	**89.67 ± 7.58**	**73.74 ± 8.82**	**93.64 ± 0.36**	**63.87 ± 1.5**
0.1	**76.77 ± 0.68**	**71.94 ± 8.11**	**57.98 ± 1.3**	**55.82 ± 1.8**
1	**65.54 ± 2.94**	**68.8 ± 1.83**	**55.34 ± 1.16**	**51.14 ± 1.44**
10	**42.54 ± 9.57**	**64.2 ± 3.4**	**46.94 ± 0.21**	**37.94 ± 1.99**
100	**16.72 ± 0.85**	**35.13 ± 1.97**	**33.49 ± 2.19**	**29.17 ± 0.95**

Data obtained from triplicate results of three experiments and shown as mean ± standard deviation (SD).

Table 3:- Percentage viability of Caco-2 cells post 48 hrs. treatment *Naja h.* venom, *Leiurus q.* venom and *Apis m*. venom and 5-FU relative to concentration.

Concentration µg/ml	Percentage viability of Caco-2 cells treated with			
	Naja h.	***Leiurus q.***	***Apis m.***	**5-FU**
0	**100**	**100**	**100**	**100**
0.01	**97.27 ± 5.18**	**92.69 ± 4.57**	**85.25 ± 0.6**	**87.56 ± 2.36**
0.1	**92.54 ± 3.45**	**91.57 ± 3.08**	**80.03 ± 3.62**	**83.48 ± 5.25**
1	**88.98 ± 8.75**	**90.78 ± 8.21**	**74.84 ± 1.69**	**73.87 ± 5.13**
10	**72.99 ± 9.97**	**87.86 ± 0.94**	**73.83 ± 7.91**	**22.1 ± 5.33**
100	**8.82 ± 1.03**	**13.26 ± 0.3**	**12.84 ± 1.21**	**11.87 ± 1.2**

Data obtained from triplicate results of three experiments and shown as mean ± standard deviation (SD).

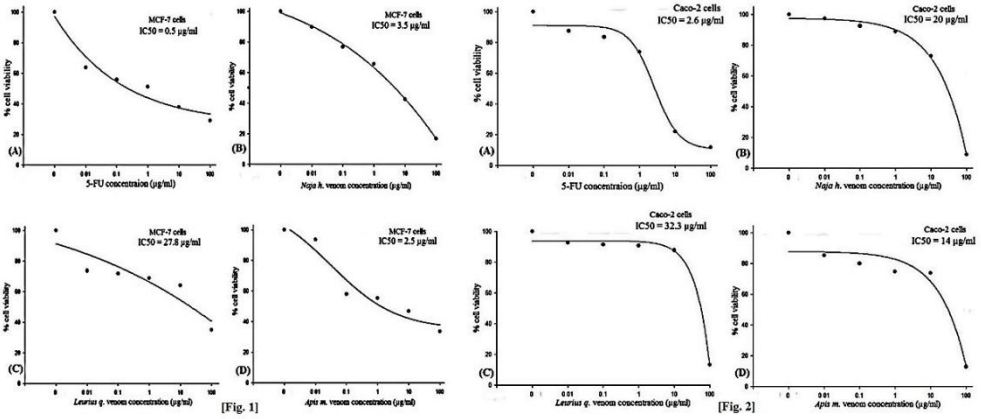


Figure 1:- Survival curve of MCF-7 cells 48 hrs. post treatment with different concentrations of venoms and anticancer drug: (A) 5-FU, (B) *Naja h.* venom (C) *Leiurus q*. venom. and (D) *Apis m.* venom.

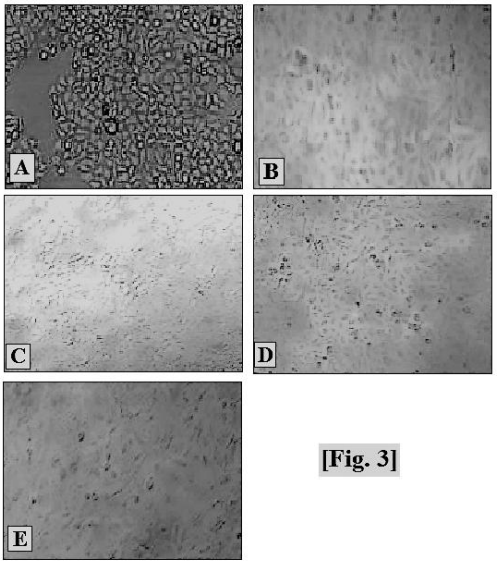
Figure 2:- Survival curve of Caco-2 cells 48 hrs. post treatment with different concentrations of venoms and anticancer drug: (A) 5-FU, (B) *Naja h.* venom (C) *Leiurus q.* venom. and (D) *Apis m.* venom.

Table 4:- IC_{50} of 5-FU, *Naja h*., *Leiurus q*. and *Apis m.* venoms treatment for 48 hrs.

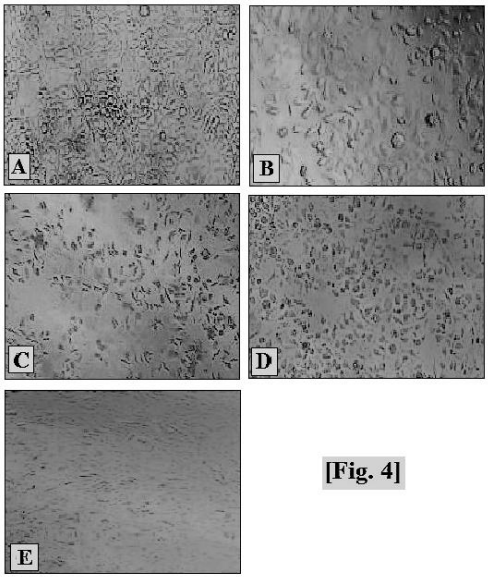
Treatment	IC_{50} (µg/ml) for 48 hrs.	
	MCF-7	**Caco-2**
Naja h. venom	**3.5**	**20**
Leiurus q. venom	**27.8**	**32.3**
Apis m. venom	**2.5**	**14**
5-FU	**0.5**	**2.6**

Morphological changes:-

Untreated MCF-7 and Caco-2 cells were well adhered and homogeneously distributed in the culture field exhibiting an epithelial shape with distinct boundaries. Morphological changes of MCF-7 and Caco-2 cells treated with IC_{50}concentrations of *Naja h*., *Leiurus q.* and *Apis m.* venoms and 5-FU for 24 hrs. were obvious whereas cells lost their characteristic appearance and started showing gradual cell shrinkage, cell rounding and detaching from the surface of tissue culture flasks. Finally it was followed by cell swelling and rupture leading to destruction of the cell sheet. It was demonstrated in Caco-2 more than MCF-7. These morphological changes were obvious in the (Figures 3 and 4).



[Fig. 3]



[Fig. 4]

Figure 3:- A photograph shows the effect of IC_{50} concentration of venoms and 5-FU on MCF-7 cells, non-treated MCF-7 cells (A) and MCF-7 cells treated with 5-FU (B) and MCF-7 cells treated with *Naja h.* venom (C) and MCF-7 cells treated with *Leiurus q*. venom (D) and MCF-7 cells treated with *Apis m*. venom (E) for 24 hrs.

Figure 4:- A photograph shows the effect of IC_{50} concentration of venoms and 5-FU on Caco-2 cells, non-treated Caco-2 cells (A) and Caco-2 cells treated with 5-FU (B) and Caco-2 cells treated with *Naja h.* venom(C) and Caco-2 cells treated with *Leiurus q*. venom (D) and Caco-2 cells treated with *Apis m*. venom (E) for 24 hrs.

Effect of *Naja h., Leiurus q.* and *Apis m.* venoms on apoptosis related genes:-

Regarding the anti-cancer potential of test venoms and the positive control drug it was recorded that P53 gene in MCF -7 and Caco-2 cells treated with the *Naja h.* and *Apis m.* venoms showed a variable significant up regulation of P53 gene post 24 hrs. cell treatment recording elevation rate in the order of 100 % in MCF-7 and 51.9% in Caco-2 for *Naja h.* venom, 50 % in MCF-7 and showed insignificant gene expression recording 15.5% in Caco-2 for *Apis m.* venom. While, *Leiurus q.* venom showed non-significant up regulation of P53 gene compared with its value in non-treated cells by 30% in MCF-7 and non-significant down regulation by 15% in Caco-2. As compared to a synthetic anticancer drug, (5-FU) showed down regulation in the order of 60% and 18.8% in MCF-7 and Caco-2 respectively and compared with negative untreated cell control (Fig. 5).

In the meantime the second pro apoptotic gene (Bax) showed a significant up regulation post MCF-7 cells treatment with *Naja h.* and *Apis m.* venoms recording 30% for both venoms compared to its value in negative cell control. While, *Leiurus q*. venom showed somewhat lower significant up regulation of Bax gene by10% compared to

negative control cells. However 5-FU drug showed a highly significant down regulation of target gene by 90% compared to non-treated cells (Fig. 5). It was noticed that there was an undetected expression rate in Caco-2 cells treated with *Naja h*. venom followed by the lowest expression rate post cellular treatment with 5-FU 0.09% compared to untreated cells. While, the up regulation rate of Bax gene was significant up regulated in *Leiurus q.* and *Apis m.* venoms treated cells recording 9% and 3.4 % respectively when compared to non-treated cells.

Regarding the Antiapoptotic gene(Bcl2): it was noticed that the Bcl-2 gene was significantly down regulated in both MCF-7 and Caco-2 cells post 24 hrs treatment with *Naja h.*, *Apis m*. and *Leiurus q.* venoms. When MCF-7 cell line was treated for 24 hrs with *Naja h.*, *Apis m*. and *Leiurus q.* venoms and 5-FU, the Bcl-2 gene down regulation rate was significant 40%, 30%, 20% and non-significant 10% respectively ($P<0.05$). While its down regulation rate was 35%, 88.84% and 97.8% when Caco-2 cell line was treated for 24 hrs. with *Leiurus q.*, *Naja h.* and *Apis m.* venoms and 5-FU respectively ($P<0.05$) (Fig 5).

Table 5:- Expression of Pro and Antiapoptotic genes in test venoms treated cells compared with 5-FU as a positive control and non-treated cells as a Negative control using RT-PCR.

Treatment	P53		Bcl-2		Bax	
	MCF-7	**Caco-2**	**MCF-7**	**Caco-2**	**MCF-7**	**Caco-2**
Naja h. venom	**2**	**1.519**	**0.6**	**0.7**	**1.3**	**____**
Leiurus q. venom	**1.3**	**0.85**	**0.8**	**0.65**	**1.1**	**1.09**
Apis m. venom	**1.5**	**1.185**	**0.7**	**0.1116**	**1.3**	**1.034**
5-FU	**0.4**	**0.812**	**0.9**	**0.22**	**0.1**	**0.00093**

Table 6:- Effect of IC_{50} concentration of Test venoms and 5-FU on the level of MDA, NO. and GSH in MCF-7 treated cells compared to negative control

Antioxidant Treatment	MDA (µM/ml) ± SE		NO. (m M/ml) ± SE		GSH (mg/ml) ± SE	
	MCF-7	**Media**	**MCF-7**	**Media**	**MCF-7**	**Media**
Control	**$0.99^{B} \pm 0.02$**	**$2.13^{A} \pm 0.02$**	**$2.66^{B} \pm 0.05$**	**$2.5^{C} \pm 0.02$**	**$13.51^{AB} \pm 0.1$**	**$10.2^{B} \pm 0.04$**
Naja h. venom	**$0.51^{D} \pm 0.03$**	**$1.38^{B} \pm 0.01$**	**$2.95^{A} \pm 0.07$**	**$3.27^{B} \pm 0.0$**	**$10.82^{C} \pm 0.1$**	**$5.93^{D} \pm 0.18$**
Leiurus q. venom	**$0.73^{C} \pm 0.03$**	**$1.38^{B} \pm 0.01$**	**$2.66^{B} \pm 0.09$**	**$3.72^{A} \pm 0.07$**	**$11.18^{C} \pm 0.42$**	**$13.89^{A} \pm 0.06$**
Apis m. venom	**$1.17^{A} \pm 0.01$**	**$1.11^{C} \pm 0.08$**	**$2.84^{AB} \pm 0.02$**	**$3.48^{AB} \pm 0.09$**	**$12.04^{BC} \pm 0.12$**	**$7.33^{C} \pm 0.13$**
5-FU	**$0.54^{D} \pm 0.02$**	**$1.1^{C} \pm 0.01$**	**$2.92^{A} \pm 0.01$**	**$3.57^{A} \pm 0.11$**	**$15.27^{A} \pm 0.08$**	**$7.53^{C} \pm 0.21$**

Table 7:- Effect of IC_{50} concentration of Test venoms and 5-FU on the level of MDA, NO. and GSH in Caco-2 treated cells compared to negative control

Antioxidant Treatment	MDA (µM/ml) ± SE		NO. (m M/ml) ± SE		GSH (mg/ml) ± SE	
	Caco-2	**Media**	**Caco-2**	**Media**	**Caco-2**	**Media**
Control	**$0.97^{C} \pm 0.04$**	**$2.25^{A} \pm 0.04$**	**$2.76^{B} \pm 0.03$**	**$3.18^{CD} \pm 0.07$**	**$15.69^{B} \pm 0.55$**	**$8.78^{C} \pm 0.26$**
Naja h. venom	**$0.63^{D} \pm 0.01$**	**$1.32^{C} \pm 0.01$**	**$2.78^{B} \pm 0.03$**	**$3.56^{AB} \pm 0.05$**	**$7.93^{C} \pm 0.43$**	**$8.33^{C} \pm 0.17$**
Leiurus q. venom	**$1.38^{B} \pm 0.06$**	**$1.57^{B} \pm 0.02$**	**$2.52^{C} \pm 0.05$**	**$3.37^{BC} \pm 0.06$**	**$18.11^{A} \pm 0.59$**	**$6.89^{D} \pm 0.09$**
Apis m. venom	**$1.32^{B} \pm 0.03$**	**$1.62^{B} \pm 0.01$**	**$3.23^{A} \pm 0.06$**	**$2.92^{D} \pm 0.06$**	**$15.18^{B} \pm 0.51$**	**$14.53^{A} \pm 0.04$**
5-FU	**$1.61^{A} \pm 0.04$**	**$1.38^{C} \pm 0.03$**	**$2.76^{B} \pm 0.04$**	**$3.79^{A} \pm 0.03$**	**$15.31^{B} \pm 0.85$**	**$11.35^{B} \pm 0.04$**

Evaluation of MDA, NO. and GSH in cells and media:-

The data recorded of *Apis m*. venom treated MCF-7 cell line revealed that there was a significant increase in MDA level ($p<0.001$) by 18.18%, while *Leiurus q.* venom showed significant decrease by 26.27%. whereas *Naja h*. venom and 5-FU had highly significant decrease of MDA level ($P<0.001$) by 48.49% and 45.46% respectively, compared to

the control. MDA level was significantly decreased post MCF-7 cell culture media treated with *Naja h*. and *Leiurus q.* venoms ($p < 0.001$), while *Apis m.* venom and 5-FU showed highly significant decrease compared to the control. In the meantime, the MDA level in Caco-2 cell line showed significant ($p < 0.001$) increase by 42.27% and 36.08% and 65.98% post treatment with *Leiurus q., Apis m.* venoms and 5-FU respectively, while *Naja h.* venom showed a significant ($P<0.001$) decreased MDA level recording 35.05% compared to the control respectively. MDA level was significantly decreased post Caco-2 cell culture media treated with *Apis m.* and *Leiurus q.* venoms ($p < 0.001$), while *Naja h*. venom and 5-FU showed highly significant decrease compared to the control.

There was no change in the NO. level in MCF-7 cells post *Leiurus q.* venom treatment. Whereas *Apis m.* venom had a non-significant increase of NO. level in MCF-7 cells by 6.77% and *Naja h.* venom and 5-FU showed significant increase in NO. level ($p<0.001$) in the MCF-7 cells by 10.9% and 9.77% respectively compared to the control. In the MCF-7 cell media *Leiurus q.* venom, 5-FU, *Apis m.* and *Naja h.* venoms showed highly significant increase in NO. level ($p<0.001$) recording 48.8% , 42.8% , 39.2% and 30.8% respectively compared to the control. While in the case of Caco-2 cells treatment with test venoms it was recorded that *Naja h.* venom and 5-FU had no change in NO. level while *Leiurus q*. venom showed significant decreased value of NO. level recording 8.7% ($p<0.001$), whereas *Apis m.* venom showed a significant elevation in NO. level recording 17.03% ($P<0.001$) compared with its values in control cells. Moreover in the Caco-2 cells media treated with 5-FU, *Naja h., Leiurus q.* venoms showed highly significant increase in NO. level ($p<0.001$) recording 19.18%, 11.95% and 5.97% respectively compared to the control. On the other hand *Apis m.* venom showed non-significant ($p<0.001$) decrease in Caco-2 media by 8.18%.

GSH level showed a non-significant elevation in MCF-7 cells treated with 5-FU by 13.03% while *Naja h., Leiurus q.* and *Apis m.* venoms recording 19.91%, 17.25% and 10.88% ($P > 0.001$) respectively. In the MCF-7 cell media 5-FU, *Apis m.* and *Naja h.* venoms showed significant decrease in GSH level recording 26.2%, 28.14% and 41.86% ($P > 0.001$) respectively. While *Leiurus q.* venom showed a significant increase in GSH level in media ($p < 0.001$) by 36.18%.

In the meantime the GSH level in Caco-2 cell line had a significant ($p < 0.001$) increase in *Leiurus q.* venom treated cells recording 15.42%. While, *Apis m.* venom and 5-FU had non-significant decrease by 3.25% and 2.42% respectively and *Naja h.* venom had a highly significant decrease recording 49.46 % ($p < 0.001$) compared to the control. Also, GSH insignificantly decreased post Caco-2 cell media treated with *Naja h*. venom recording 5.13% ($p > 0.001$). While *Leiurus q.* venom showed significant decrease in GSH level by 21.53% ($P <0.001$). On the contrary, there was a significant elevation of GSH level detected post cell treatment with 5-FU as

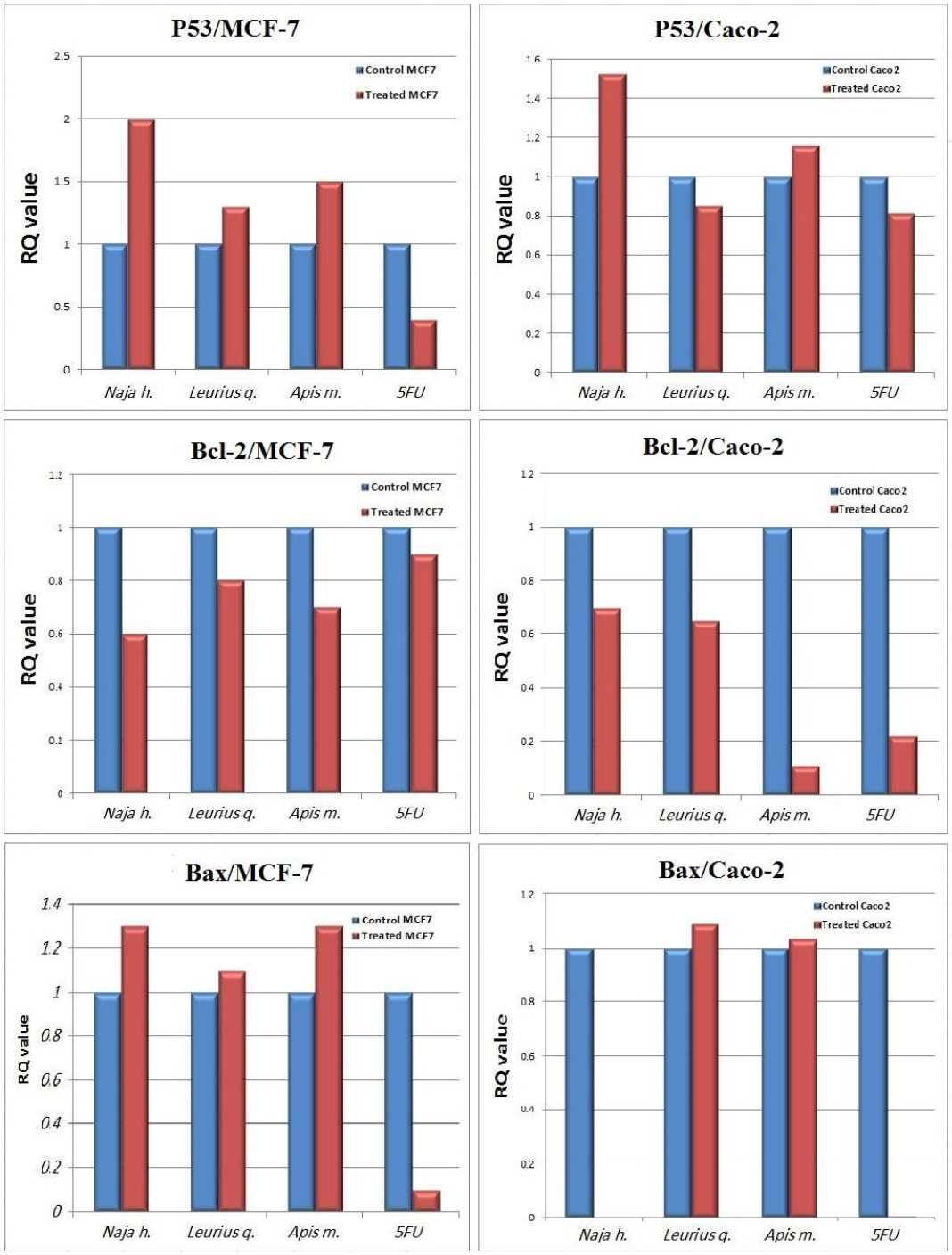


Figure 5:- Evaluation of p53, BAX and Bcl2 mRNA expression levels 24h Post MCF-7 and Caco2 cells treatment with IC_{50} of *Naja h., Leiurus q. Apis m.* and 5-FU.

well as *Apis m.* venom recording 29.27% and 65.49% respectively ($p < 0.001$) compared to the control.

Discussion:-

Cancer is primarily a genetic disease and is one of the most reasons of death worldwide. Cancer has wide enormous chances to be controlled. There is a vital need to discover a better treatment because of its fast spreading rate in the present era. Recently a significant movement has been made in the direction of understanding suggested hallmarks of cancer growth and treatment. Anticancer drug development from natural resources is offered all over the world.

Venoms of several animal species including snakes, scorpions and bees as well as their biochemical derivatives have shown therapeutic potential against cancer as Captopril and Escozul **(Bryan, 2009; Lorenzo *et al.*, 2012).** In the near future, these molecules may become well-known anticancer drugs.

In the present study, we traced the apoptotic pattern and anti-proliferation factors to evaluate the probable mechanism of action of *Naja h.*, *Leiurus q*. and *Apis m.* venoms on breast and colon cancer cells. This is done by evaluating the anticancer potentials through measuring the pro-apoptotic and anti-apoptotic genes and related antioxidants profile in treated and non-treated cells compared to the effect of 5-FU as a synthetic anti-cancer drug.
Compatible with previous studies, animal venoms have a cytotoxic and anti-proliferation effect on cancer cells **(Zargan *et al.*, 2011$_b$; Zargan *et al.*, 2011$_c$; Park *et al.*, 2011; Ebrahim *et al.*, 2016).**

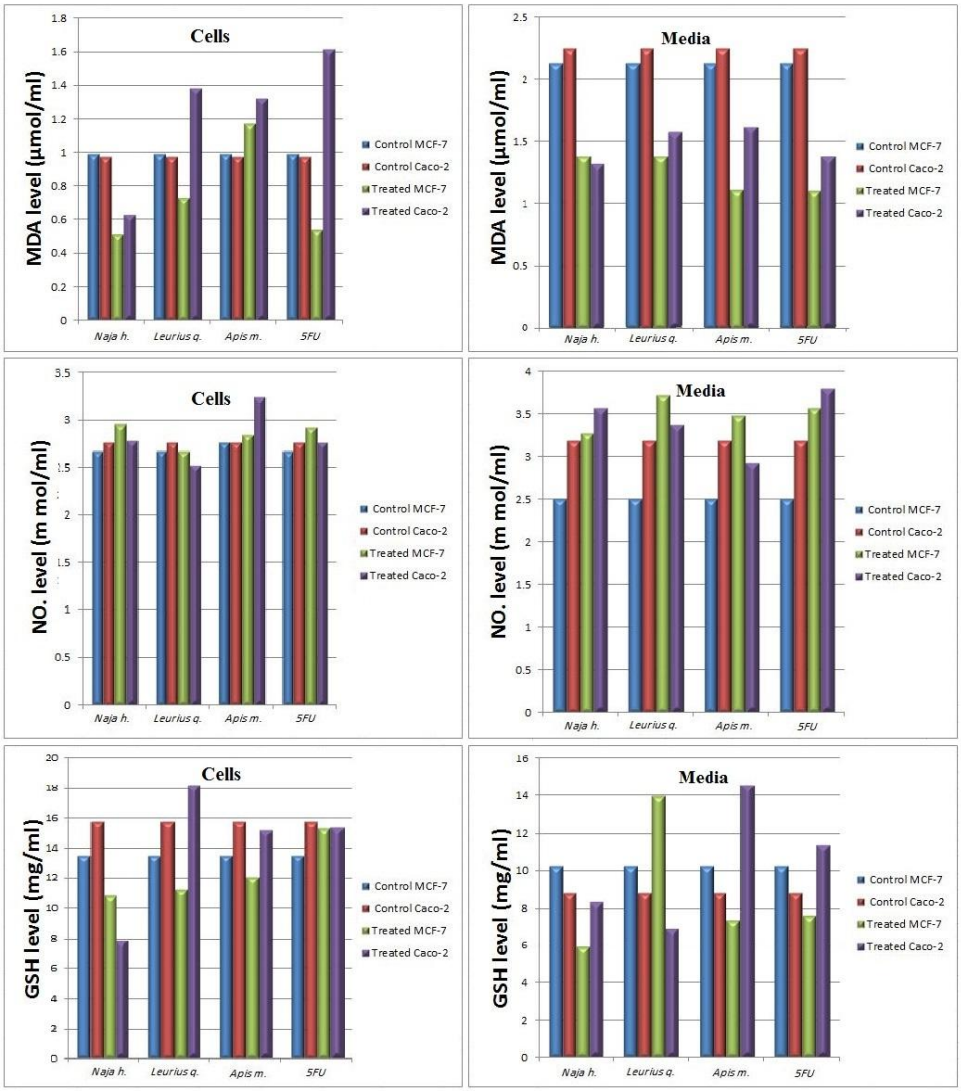


Figure 6:- Bar diagram showing the levels of MDA, NO. and GSH in the MCF-7 and Caco-2 cells and media treated with *Naja h., Leiurus q*. and *Apis m.* venoms and 5-FU exposure for 24 hrs. Data are represented the mean ± SE.

In another study, *Naja h.* and *Apis m*. venoms and their derivatives showed cytotoxic anti proliferative effect on breast and lung cancer cell lines **(El Sharkawi *et al.*, 2015).** Another important finding is *Leiurus q.* venom inhibits the proliferation of breast and prostate cancer cells **(Omran, 2003).**In addition, the Al-Asmari *et al.* study reported that colon and breast cancer cells are sensitive to *Leiurus q.* venom **(Al-Asmari *et al.*, 2016a).**

Variation in the cytotoxic concentration between the three venoms may be attributed to the variations of venom constituents. In the present study data regarding the morphological findings accompanied with reduction in cell viability was in accordance with data reported by **(Akbari and Javar, 2013)** assuring that 50% of MCF-7 cells proliferation has been inhibited by 0.38 µg/ml of 5-FU after 48 hours**.** Also, a study conducted by **(Gomez-Monterrey *et al.*, 2013)** reported that the proliferation of human colon cancer cells in response to 5-FU, showed that the IC_{50} of 5-FU on Caco-2 cell line after 48 hours is 46 µM/ ml, which is equal to 5.9 µg/ml. The difference between the results of this study and the previous studies may be due to the difference in evaluation protocols, and species and habitat of used animal as a source of venom.

It is known that oxidative stress occurs as a defense mechanism which is imbalanced by free radicals. This imbalance can be caused by either increased free radical formation or decreased antioxidant capacity **(Birben *et al.*, 2012).** The mitochondria plays a key role in cell apoptosis and ROS production. ROS have critical actions on both DNA and proteins. The increasing level of ROS causes receptors activation as Fas receptor. ROS oxidize the mitochondrial pores and disrupt the mitochondrial membrane potential that leads to cytochrome C release, mitochondrial membrane lipid peroxidation, membrane fluidity reduction, membrane lipid degradation and *etc.* **(Simon *et al.*, 2000).**

GSH is one of the main intracellular antioxidants with several biological functions. One of its important functions is to protect against ROS-caused oxidative damage. Also, MDA level provides other marks of oxidative stress association in venom toxicity. Moreover nitric oxide level which is elevated in cancer cells has antitumor activity, but at a lower concentration it stimulates tumor growth (**Jenkins *et al.*, 1995**). So, this study provides evidence supporting the theory that GSH, NO. and MDA play an important role in genotoxicity of venom treatment.

Regarding the anticancer activity of test venoms based on the elevated or unelevated levels of antioxidant, our data was in agreement with others. For example in Al-Quraishy *et al.* study, it showed that rats injected with *Naja h.* venom had a significant increase of the NO. level in serum and liver tissue by 42.97% and 49.23% respectively **(Al-Quraishy *et al.*, 2014).** Similar observation was reported by **(Dkhil *et al.*, 2014)** that *Naja h.* venom increased the NO. level in kidney tissue by 82.2%**.** Snake venom of *Walterinnesia a.* was found to have an increasing effect on NO. level in breast and prostate cancer tissues by 130% and 100% respectively **(Badr *et al.*, 2013).** The mechanism of how snake venom increases the nitric oxide concentration was thought to be by up regulating of iNOS gene expression (**Al-Quraishy *et al.*, 2014; Dkhil *et al.*, 2014**). Al-Asmari and co-workers found that different snake venoms could cause apoptosis in colon and breast cancer cell lines through increasing ROS **(Al-Asmari *et al.*, 2016_b).** Dkhil *et al.* found that *Naja h.* venom induced renal toxicity to rat kidney tissue through decreasing GSH content by 41.02% and through the reduction of GR, GST, and GPx genes expression **(Dkhil *et al.*, 2014)**. In another research, the reduced GSH in liver tissue of envenomed rats with $LD_{50\%}$of *Naja h.* venom is due to reduction of GR expression **(Al-Quraishy *et al.*, 2014)**. *Naja h.* venom induced significant increase in lipid peroxidation in the liver and kidney tissues, but not in the heart tissue **(Moneim *et al.*, 2015)**. On the opposite view, *Naja h.* venom induced high lipid peroxidation with $LD_{50\%}$ to rat kidney and liver tissues and serum by 78.54%, 18.8% and 34.54% respectively **(Dkhil *et al.*, 2014; Al-Quraishy *et al.*, 2014).**

Also, *Leiurus q.* venom showed a variable effect in antioxidant level and our data was compatible with others. According to Zargan and his team studied the *Odontobuthus d.* venom on MCF-7 cell line and found that 50µg/ml and 100µg/ml scorpion venom increased the NO. level. Also, its concentration in supernatant is increased by 31.23% and 47.95% respectively, when compared to control. In another study of Zargan team but on SH-SY5Y cell line, found that the scorpion venom increased the NO. concentration in supernatant by two fold or four fold by 50µg/ml and 100µg/ml. Another research of this team found that the venom of scorpion *Androctonus c.*(50µg/ml) increased the NO. concentration in supernatant of MCF-7 and SH-SY5Y cell lines by 38.89% and 324.26% respectively **(Zargan *et al.*, 2011_a; Zargan *et al.*, 2011_b; Zargan *et al.*, 2011_c).** In previous studies, the mechanism of how scorpion venom increases the extracellular nitric oxide concentration is still unknown. Scorpion venom of *Odontobuthus d.* decreased the GSH level in human breast cancer cell line MCF-7 (**Zargan *et al.*, 2011_b).** Envenomed rats with *Leiurus q.* venom had an elevated GSH level in heart and lung tissues **(Fatani *et al.*, 2006)**.

Human lung cancer cell line (NCI-H358) treated with *Androctonus a.* venom showed a significant decrease in GSH level by 16.66 % **(Béchohra *et al.*, 2016).** Envenomed rats with *Leiurus q.* venom had an elevated MDA level in heart tissue but no change in lung tissue **(Fatani *et al.*, 2006)**. Human lung cancer cell line (NCI-H358) treated with *Androctonus a.* venom showed a significant increase in MDA level **(Béchohra *et al.*, 2016).**

Most previous studies described that bee venom decreased the NO content and NOS mRNA expression as in BV-2 cell line study **(Han *et al.*, 2007).** The bee venom has a critical role in increasing the intracellular Ca^{++} **(Tu *et al.*, 2008).** Ca^{++} has a role in NO. production as described previously. Furthermore this study of bee venom had neuroprotective effects in mice brain; bee venom neutralized the GSH level in brain tissue **(Khalil *et al.*, 2015).** And Melittin; the most predominant component of bee venom, slightly reduced the GSH levels in human peripheral blood lymphocytes **(Gajski *et al.*, 2012; Gajski *et al.*, 2016).** By studying bee venom neuroprotective effects in mice brain was found that bee venom increases the MDA levels in brain by 77.7% **(Khalil *et al.*, 2015).** Bee venom increased the MDA levels in human peripheral blood lymphocytes by 37.21% **(Gajski *et al.*, 2012).** Also melittin increased the MDA levels in HPBLs cells **(Gajski *et al.*, 2016).**

Besides, it was reported that 5-FU induced endogenous nitric oxide in BEL-7402 human liver carcinoma by 173%. *In vivo* and *in vitro* studies of 5-FU induces the expression of iNOS that might be by induction of cytokines **(Yin *et al.*, 2007)**. On the other hand, 5-FU had no effect on nitric oxide production in the DLD-1 colon cancer cell line but also inhibited the cell ability to express NOS mRNA **(Jin *et al.*, 1996).** In patients with colorectal cancer, GSH levels in plasma and erythrocytes decreased and the lipid peroxidation levels increased after 5-FU treatment **(Koçer and Nazıroğlu, 2013).** In patients with breast cancer, the GSH levels increased and the lipid peroxidation levels decreased after 5-FU treatment **(Suhail *et al.*, 2012).**

Fighting tumor cells by most anticancer strategies now used in clinical oncology, for example, chemotherapy, radiation, gene therapy or immunotherapy, has been linked to activation of apoptosis signal transduction pathways in cancer cells such as the intrinsic and/or extrinsic pathway. Venoms that have anticancer effect may induce apoptosis by extrinsic and/or intrinsic pathway **(Jo *et al.*, 2012; Song *et al.*, 2012).** The intrinsic signaling pathway of apoptosis has reported a non-receptor mediated stimuli and production of intracellular signals that act directly on targets within the cell. The stimuli that initiate the intrinsic pathway produce the intracellular signals may be radiation, oxidant agent and toxins. This stimuli depends on the change in the inner mitochondrial membrane and the release of a pro-apoptotic protein such as cytochrome C that activates caspases especially caspase 9 **(Fulda and Debatin, 2006).**

The control and regulation of apoptotic mitochondrial pathway occurs through members of the Bcl-2 family proteins that can either be pro-apoptotic or anti-apoptotic. The tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family Proteins **(Adams and Cory, 2007).** The tumor suppressor gene p53 is activated in the nucleus by DNA damage or oxidative stress. The resulting increase in p53 level leads either to the induction of the cell cycle arrest or apoptosis that suppress the tumor growth **(Kruiswijk *et al.*, 2015).** Bcl-2 is an anti-apoptotic protein that prevents cell death and apoptosis whereas BAX is a pro-apoptotic protein that stimulates apoptosis **(Adams and Cory, 2007).**

In agreement with the present study, initiation of apoptosis is explained by up regulation of both p53 and Bax genes expression and down regulation of Bcl-2 gene expression. In other studies, snake venom from *Vipera lebentina turnica* induced apoptotic cell death of human lung cancer cells via increasing of caspase 3, Bax, p21 and p53, but decreasing cIAP and Bcl-2 expressions **(Lee *et al.*, 2015).** The East-Asian scorpion *Buthus martensi Karsch* on human prostate cancer cells has an apoptotic effect by stimulating the expression of p27 and Bax while decreasing cyclin E and Bcl-2 **(Zhang *et al.*, 2009).** Bee venom inhibits the cell growth and apoptosis of the human ovarian cancer cells through the inhibition of STAT3 and Bcl2 proteins and increasing pro-apoptotic proteins including caspase-3, 8, and Bax **(Jo *et al.*, 2012).**

The mRNA expression of apoptosis related genes in MCF-7 cells treated with *Apis m.* venom were more affected than these in Caco-2 cells. In MCF-7, P53 and Bax mRNA expression were increased by 50% and 30% respectively but Bcl-2 mRNA expression was decreased by 30%, when compared to untreated cells. In Caco-2, P53 and Bax mRNA expression were increased by 15.5% and 3.4% respectively but Bcl-2 mRNA expression was decreased by 88.84%, when compared to untreated cells. In the study of bee venom neuroprotective effects in mice brain, it was found that bee venom increases the mRNA levels of caspase-3, Bcl-2 and Bax genes in brain tissues of mice **(Khalil**

***et al.,* 2015)**. Furthermore bee venom induced apoptosis in human breast cancer MCF-7 cells, human cervical epidermoid carcinoma Ca Ski cells, human Lung NCI-H1299 cells, human leukemic U937 cells and human bladder cancer TSGH-8301 cells through increasing in P53 and Bax expression and decreasing in Bcl-2 expression **(Jang *et al.,* 2003; Ip *et al.,* 2008a; Ip *et al.,* 2008b; Ip *et al.,* 2012).** Also, Melittin up regulated the mRNA levels of TP53 (1.4-fold), caspase-7 (2.36-fold), Bcl-2 (2.95-fold) and Bax genes (3-fold) in human peripheral blood lymphocytes **(Gajski *et al.,* 2016).**

In agreement with the present study, initiation of apoptosis is explained by up regulation of both p53 and Bax genes expression and down regulation of Bcl-2 gene expression. In other studies, snake venom from *Vipera lebentina turnica* induces apoptotic cell death of human lung cancer cells via increasing of caspase 3, Bax, p21 and p53, but decreasing cIAP and Bcl-2 expressions **(Lee *et al*., 2015).**

It was reported by **(Ayman *et al.,* 2017)** that *Cerastes cerastes* venom showed anti-cancer potential against both prostate (PC3) and colon (Caco-2) cancer cell lines and their potential was significantly elevated under the effect of BCG derived PPD protein in a concentration dependent way. Besides, it was reported by **(Abd-Elrahem *et al.,* 2017)** that propolis, PLA2 and *Apis m.* venom showed anticancer potential in a variable way proving that anticancer activity is P53 gene pathway dependent. Also, it was recorded by **(Ayman *et al.,* 2017)** and **(Abd-Elrahem *et al.,* 2017)** that The main phase of cell cycle arrest was G2/M in both cancer cell lines, with a minor S-phase arrest. According to our study results, there is an increase in ROS level in human colon cancer Caco-2 cells more than human breast cancer MCF-7 cells in response to *Apis m.* venom treatment. The elevated ROS level is through increasing of NO. and MDA levels and decreasing of GSH level. GSH, NO. and MDA are well-established signs of oxidative stress and these results might have a role in bee venom cytotoxicity and apoptotic effect. These results are compatible with previous studies that observed bee venom treatment induced ROS production in human cervical epidermoid carcinoma Ca Ski cells **(Ip *et al.,* 2008b)**, human bladder cancer TSGH-8301 cells **(Ip *et al.,* 2012)** in addition to an increase in hydroxyl radicals and hydrogen peroxides production in human melanoma A2058 cells **(Tu *et al.,* 2008).** While in mice, bee venom induced an elevation in serum MDA and NO **(Prado *et al.,* 2010)** and an elevation in brain MDA and a decrease in brain GSH **(Khalil *et al.,* 2015).** Also, previous studies that described the mode of action of bee venom in cancer treatment found that bee venom induced apoptosis in cancer cells (breast, cervical, prostate and ovarian) through many factors. These may be through ROS production, dysfunction of the mitochondrial membrane potential, suppression of the activated NF-κB, release of cytochrome C from mitochondria, increase in the levels of caspases as caspase-3,8,9, activates DRs as DR3, DR4 and DR6 and increases in the levels of Fas, p53, p21, iNOS, COX-2 and Bax, but decreases in the level of Bcl-2 and STAT3 genes expression. Also Bee venom induces cell cycle arrest and necrosis in some cells **(Ip *et al.,* 2008$_a$; Ip *et al.,* 2008$_b$; Park *et al.,* 2011; Jo *et al.,* 2012 ; Ayman *et al.,* 2017; Abd-Elrahem *et al.,* 2017).**

Regarding our data concerning the mRNA expression of apoptosis related genes of MCF-7 treated with *Leiurus q.* venom that was more sensitive than these of Caco-2, our result was in agreement with Alyan *et al.* study that studied the effect of a safe concentrations of crude and pure *Leiurus q.* venom on MCF-7 and Caco-2 cell lines. This study found that *Leiurus q.* venom had no effect on caco-2 cells but showed a slight effect on mcf-7 apoptosis related genes **(Alyan *et al., 2014)*.** While Scorpion venom of *Rhopalurus j.* had dissimilar effect on different cancer cell types. Cervix adenocarcinoma cells (Hela) treated with scorpion venom had a significant increase in p53 and bax expression but Bcl-2 depletion. Also, Scorpion venom has an effect on lung carcinoma cells (A549) through a significant decrease in p53 and bcl-2 but no change in Bax expression **(Díaz-García *et al.,* 2013).**

Furthermore MCF-7 cells treated with *Naja h.* venom were more sensitive than these of Caco-2 in the mRNA expression of apoptosis related genes. MCF-7, P53 and Bax genes were up regulated by 100% and 30% respectively but Bcl-2 mRNA expression was down regulated by 40%, when compared to untreated cells, while in Caco-2 cells, P53 mRNA expression was increased by 51.9% while Bcl-2 mRNA expression was decreased by 30%, compared to untreated cell control. On the other hand there is no expression in Bax mRNA. MCF-7 cells that was treated with safe concentration of *Naja h.* venom had no effect on the apoptotic related genes. On the other hand, Caco-2 cells had an effect on P53 and Bax and no effect on Bcl-2 **(Alyan *et al.,* 2014)**. *Also, Naja h.* venom up regulated Bax but down regulated Bcl2 protein levels in liver, heart, and kidney tissues **(Moneim *et al.,* 2015)**.

5-FU treated MCF-7 cells showed a down regulation in Bcl-2 and P53 gene expression by 10% and 60% respectively. MCF-7 treated with $IC_{50\%}$ of 5-FU induced apoptosis by slightly down regulation of Bcl-2 with slight up regulation of P53 genes **(Hernández-Vargas *et al.,* 2006).** Also, the effect of 5-FU on Caco-2 cells down

regulated P53, Bcl-2 and Bax by 18.8%, 97.8% and 99.91% respectively. In the study that evaluated the apoptosis in various colon cancer cell lines treated with 5-FU, 5-FU induced the lowest Bax expression and not changing in regulation of mutant P53but a variation in Bcl-2 expression pattern **(Nita *et al.*, 1998).** Also, it was reported by **(Chan *et al.*, 2008)** that 5-FU could induce apoptosis in absence of p53 in colon cancer cell lines**.**

The above results suggest that the half lethal dose (IC_{50}) of snake *(Naja h.),* scorpion *(Leiurus q.)* and bee *(Apis m.)* venoms had anticancer potentials on human breast and colon cancer cells and this is positively related to the antioxidant profile and apoptotic gene expression. Hence, we concluded that tested venoms possesses many bioactive substances, especially peptides and proteases that bind with high affinity to physiological targets and can be trapped for therapeutic purposes in the near future.

Finally, it can be concluded that snake *(Naja h.),* scorpion *(Leiurus q.)* and bee *(Apis m.),* venoms have anticancer potentials on human breast and colon cancer cells and this is positively related to the antioxidant profile and apoptotic gene expression.

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References:-

1. **Abd-Elrahem, H. T.; El-Fiky, A. A.; Bakkar, A.; Mohamed, A. F. (2017):** Honey Bee, Propolis and Bee Venom Derivatives Induced Apoptosis in Colorectal Cancer Cell Line HCT-116. *Inventi Rapid: Mol. Pharmacol*., 17(2):1-7.
2. **Adams, J. M. and Cory, S. (2007):** The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene*, 26(9): 1324-1337.
3. **Akbari, R. and Javar, H. A. (2013):** Efficacy of Capecitabine and 5-Fluorouracil (5-FU) on the human breast cancer cell line (MCF7) – effect of concentration. *Am. J. Res. Commun.*, 1(16): 75-91.
4. **Al-Asmari, A. K.; Islam, M. and Al-Zahrani, A. M. (2016a**): *In vitro* analysis of the anticancer properties of scorpion venom in colorectal and breast cancer cell lines. *Oncol. Lett.*, 11(2): 1256-1262.
5. **Al-Asmari, A. K.; Riyasdeen, A.; Al-Shahrani, M. H. and Islam, M. (2016b):** Snake venom causes apoptosis by increasing the reactive oxygen species in colorectal and breast cancer cell lines. *Onco.Targets. Ther.*, 9(16), 6485-6498.
6. **Al-Quraishy, S.; Dkhil, M. A. and Moneim, A. E. A. (2014):** Hepatotoxicity and oxidative stress induced by *Naja haje* crude venom. *J. Venom. Anim. Toxins Incl. Trop. Dis*., 20(1): 42-52.
7. **Alyan, M. S.; Shalaby, M. A.; El-Sanousi, A. A.; Fahmy, A.; El-Sayed, M. and Shebl, R. I. (2014):** Antiviral and Anticancer Potentials of Snake and Scorpion Venom Derivatives. *Inventi Rapid: Mol. Pharmacol*., 2014(2): 1-11.
8. **Ayman, M.; Mohmed, A. F.; Bakaar, A.; Shebl, R. l.; Sabatier, J. M. (2017):** The Bacillus Calmette-Guérin Derived Purified Protein (PPD) Potentiates *In-Vitro* Anti-Cancer Activity of *Cerastes cerastes* Snake Venom in Colon and Prostate Cancer Cells. *Inventi Rapid: Mol. Pharmacol.*, 17(1):1-10.
9. **Badr, G.; Al-Sadoon, M. K. and Rabah, D. M. (2013):** Therapeutic efficacy and molecular mechanisms of snake (*Walterinnesia aegyptia*) venom-loaded silica nanoparticles in the treatment of breast cancer-and prostate cancer-bearing experimental mouse models. *Free Radic. Biol. Med.*, 65(13): 175-189.
10. **Badr, G.; Sayed, D.; Maximous, D.; Mohamed, A. O. and Gul, M. (2014):** Increased susceptibility to apoptosis and growth arrest of human breast cancer cells treated by a snake venom-loaded silica nanoparticles. *Cell. Physiol. Biochem*., 34(5): 1640-1651.
11. **Barrera, G.; Pizzimenti, S. and Dianzani, M. U. (2008):** Lipid peroxidation: control of cell proliferation, cell differentiation and cell death. *Mol. Aspects Med*., 29(1): 1-8.
12. **Béchohra, L.; Laraba-Djebari, F. and Hammoudi-Triki, D. (2016):** Cytotoxic activity of *Androctonus australis* hector venom and its toxic fractions on human lung cancer cell line. *J. Venom. Anim. Toxins Incl. Trop. Dis*., 22(16): 14-29.
13. **Birben, E.; Sahiner, U. M.; Sackesen, C.; Erzurum, S. and Kalayci, O. (2012):** Oxidative stress and antioxidant defense. *World Allergy Organ. J*., 5(1): 9-19.
14. **Bryan, J. (2009):** From snake venom to ACE inhibitor-The discovery and rise of captopril. *Pharm. J.*, 282(7548): 455-459.

15. **Bunz, F.; Hwang, P. M.; Torrance, C.; Waldman, T.; Zhang, Y.; Dillehay, L.; Williams, J.; Lengauer, C.; Kinzler, K. W. and Vogelstein, B. (1999):** Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J. Clin. Invest*., 104(3): 263-269.
16. **Chan, J. Y.; Phoo, M. S.; Clement, M. V.; Pervaiz, S.; and Lee, S. C. (2008):** Resveratrol displays converse dose-related effects on 5-fluorouracil-evoked colon cancer cell apoptosis: the roles of caspase-6 and p53. *Cancer Boil. Ther*., 7(8): 1305-1312.
17. **Corrêa, M. C.; Maria, D. A.; Moura-da-Silva, A. M.; Pizzocaro, K. F. and Ruiz, I. R. (2002):** Inhibition of melanoma cells tumorigenicity by the snake venom toxin jararhagin. *Toxicon*, 40(6): 739-748.
18. **D'Suze, G.; Rosales, A.; Salazar, V. and Sevcik, C. (2010):** Apoptogenic peptides from *Tityus discrepans* scorpion venom acting against the SKBR3 breast cancer cell line. *Toxicon*, 56(8): 1497-1505.
19. **Debin, J. A.; Maggio, J. E. and Strichartz, G. R. (1993):** Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. *Am. J. Physiol. Cell Physiol*., 264(2): C361-C369.
20. **Díaz-García, A.; Morier-Díaz, L.; Frión-Herrera, Y.; Rodríguez-Sánchez, H.; Caballero-Lorenzo, Y.; Mendoza-Llanes, D.; Riquenes-Garlobo, Y. and Fraga-Castro, J. A. (2013):***In vitro* anticancer effect of venom from Cuban scorpion *Rhopalurus junceus* against a panel of human cancer cell lines. *J. Venom Res*., 4(13): 5-12.
21. **Dimmeler, S.; Haendeler, J.; Nehls, M. and Zeiher, A. M. (1997):** Suppression of apoptosis by nitric oxide via inhibition of interleukin-1β–converting enzyme (ice)-like and cysteine protease protein (cpp)-32–like proteases. *J. Exp. Med.*, 185(4): 601-608.
22. **Dkhil, M. A.; Al-Quraishy, S.; Farrag, A. R. H.; Aref, A. M.; Othman, M. S. and Moneim, A. E. A. (2014):** Oxidative stress and apoptosis are markers in renal toxicity following Egyptian cobra (*Naja haje*) envenomation. *Pak. J. Zool*., 46(6): 1719-1730.
23. **Draper, H. H. and Hadley, M. (1990**): A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *Xenobiotica*, 20(9): 901-907.
24. **Ebrahim, K.; Vatanpour, H.; Zare, A.; H Shirazi, F. and Nakhjavani, M. (2016):** Anticancer Activity a of Caspian Cobra (*Naja naja oxiana*) snake Venom in Human Cancer Cell Lines Via Induction of Apoptosis. *Iran. J. Pharm. Res*., 15, 101-112.
25. **El Sharkawi, F. Z.; Saleh, S. S. and El Sayed, A. F. M. (2015):** Potential anti cancer activity of snake venom, bee venom and their components in liver and breast carsenoma. *Int. J. Pharm. Sci. Res*., 6(8): 3224-3235.
26. **Ellman, G. L. (1959):** Tissue sulfhydryl groups. *Arch. Biochem. Biophys*., 82(1): 70-77.
27. **Fatani, A. J.; Al-Zuhair, H. A.; Yaquob, H. I.; Abdel-Fattah, A. A.; El-Sayed, M. I. and El-Sayed, F. A. (2006):** Protective effects of the antioxidant Ginkgo biloba extract and the protease inhibitor aprotinin against *Leiurus quinquestriatus* venom-induced tissue damage in rats. *J. Venom. Anim. Toxins Incl. Trop. Dis*., 12(2): 255-275.
28. **Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D. and Bray, F. (2015):** Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer*, 136(5): E359-E386.
29. **Fesik, S. W. (2005**): Promoting apoptosis as a strategy for cancer drug discovery. *Nat. Rev. Cancer*, 5(11): 876-885.
30. **Franco, R., and Cidlowski, J. A. (2009):** Apoptosis and glutathione: beyond an antioxidant. *Cell Death Differ*., 16(10): 1303-1314.
31. **Fulda, S. and Debatin, K. M. (2006):** Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*, 25(34): 4798-4811.
32. **Gajski, G.; Čimbora-Zovko, T.; Rak, S.; Rožman, M.;Osmak, M. and Garaj-Vrhovac, V. (2014):** Combined antitumor effects of bee venom and cisplatin on human cervical and laryngeal carcinoma cells and their drug resistant sublines. *J. Appl.Toxicol.*, 34(12): 1332-1341.
33. **Gajski, G.; Domijan, A. M. and Garaj-Vrhovac, V. (2012):** Alterations of GSH and MDA levels and their association with bee venom-induced DNA damage in human peripheral blood leukocytes. *Environ. Mol. Mutagen*., 53(6): 469-477.
34. **Gajski, G.; Domijan, A. M.; Žegura, B.; Štern, A.; Gerić, M.; Jovanović, I. N.; Jovanovi, I. N.; Vrhovac, I.; Maduni, J.; Breljak, D.; Filip, M. and Garaj-Vrhovac, V. (2016):** Melittin induced cytogenetic damage, oxidative stress and changes in gene expression in human peripheral blood lymphocytes. *Toxicon*, 110(16): 56-67.
35. **Galvez, A.; Gimenez-Gallego, G.; Reuben, J. P.; Roy-Contancin, L.; Feigenbaum, P.; Kaczorowski, G. J. and Garcia, M. L. (1990):** Purification and characterization of a unique, potent, peptidyl probe for the high

conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. *J. Biol. Chem*., 265(19): 11083-11090.

36. **Garcia-Calvo, M.; Leonard, R. J.; Novick, J.; Stevens, S. P.; Schmalhofer, W.; Kaczorowski, G. J. and Garcia, M. L. (1993):** Purification, characterization, and biosynthesis of margatoxin, a component of *Centruroides margaritatus* venom that selectively inhibits voltage-dependent potassium channels. *J. Biol. Chem*., 268(25): 18866-18874.
37. **Giménez-Bonafé, P.; Tortosa, A. and Pérez-Tomás, R. (2009):** Overcoming drug resistance by enhancing apoptosis of tumor cells. *Curr. Cancer Drug Targets*, 9(3): 320-340.
38. **Gomes, A.; Bhattacharjee, P.; Mishra, R.; Biswas, A. K.; Dasgupta, S. C. and Giri, B. (2010):** Anticancer potential of animal venoms and toxins. *Indian J. Exp. Biol*., 48,(2): 93-103.
39. **Gomez-Monterrey, I.; Campiglia, P.; Scognamiglio, I.; Vanacore, D.; Dicitore, A.; Lombardi, A.; Caraglia, M.;Novellino, E. and Stiuso, P. (2013):** DTNQ-Pro, a mimetic dipeptide, sensitizes human colon cancer cells to 5-fluorouracil treatment. *J. Amino Acids*, (13): 1-7.
40. **Goudet, C.; Chi, C. W. and Tytgat, J. (2002):** An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensi Karsch. Toxicon*, 40(9): 1239-1258.
41. **Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S. and Tannenbaum, S. R. (1982):** Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. *Anal. Biochem.*, 126(1): 131-138.
42. **Gupta, S. D.; Gomes, A.; Debnath, A.; Saha, A. and Gomes, A. (2010):** Apoptosis induction in human leukemic cells by a novel protein Bengalin, isolated from Indian black scorpion venom: through mitochondrial pathway and inhibition of heat shock proteins. *Chem. Biol. Interact*., 183(2): 293-303.
43. **Haddad, J. J. (2004):** Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *Int. Immunopharmacol*., 4(4): 475-493.
44. **Han, S. M.; Lee, K. G.; Yeo, J. H.; Kweon, H. Y.; Woo, S. O.; Lee, M. L.; Baek, H. J.; Kim, S. Y. and Park, K. K. (2007):** Effect of honey bee venom on microglial cells nitric oxide and tumor necrosis factor-α production stimulated by LPS. *J. Ethnopharmacol*., 111(1): 176-181.
45. **Hernández-Vargas, H.; Ballestar, E.; Carmona-Saez, P.; von Kobbe, C.; Bañón-Rodríguez, I.; Esteller, M.; Moreno-Bueno, G. and Palacios, J. (2006):** Transcriptional profiling of MCF7 breast cancer cells in response to 5-Fluorouracil: Relationship with cell cycle changes and apoptosis, and identification of novel targets of p53. *Int. J. Cancer*, 119(5): 1164-1175.
46. **Higuchi, Y. (2004):** Glutathione depletion-induced chromosomal DNA fragmentation associated with apoptosis and necrosis. *J. Cell. Mol. Med*., 8(4): 455-464.
47. **Harvey, A. L. (2014):** Toxins and drug discovery. *Toxicon*, 92, 193-200.
48. **Houghton, P.; Fang, R.; Techatanawat, I.; Steventon, G.; Hylands, P. J. and Lee, C. C. (2007):** The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods*, 42(4): 377-387.
49. **Ip, S. W.; Chu, Y. L.; Yu, C. S.; Chen, P. Y.; Ho, H. C.; Yang, J. S.; Huang, H. Y.; Chueh, F. S. and Chung, J. G. (2012):** Bee venom induces apoptosis through intracellular Ca2+-modulated intrinsic death pathway in human bladder cancer cells. *Int. J. Urol*., 19(1): 61-70.
50. **Ip, S. W.; Liao, S. S.; Lin, S. Y.; Lin, J. P.; Yang, J. S.; Lin, M. L.; Chen, G. W.; Lu, H. F.; Lin, M. W.; Han, S. M. and Chung, J. G. (2008a):** The role of mitochondria in bee venom-induced apoptosis in human breast cancer MCF7 cells. *In Vivo*, 22(2): 237-245.
51. **Ip, S. W.; Wei, H. C.; Lin, J. P.; Kuo, H. M.; Liu, K. C.; Hsu, S. C.; Yang, J. S.; Mei-Dueyang; Chiu, T. H.; Han, S. M. and Chung, J. G. (2008b):** Bee venom induced cell cycle arrest and apoptosis in human cervical epidermoid carcinoma Ca Ski cells. *Anticancer Res*.,28(2A): 833-842.
52. **Jain, K. K. (2014):** Molecular biology of cancer in Applications of biotechnology in oncology. New York: *Springer*, pp. 1-22.
53. **Jang, M. H.; Shin, M. C.; Lim, S.; Han, S. M.; Park, H. J.; Shin, I.; Lee, J. S.; Kim, K. A.; Kim, E. H. and Kim, C. J. (2003):** Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. *J. Pharmacol. Sci.*, 91(2): 95-104.
54. **Jenkins, D. C.; Charles, I. G.; Thomsen, L. L.; Moss, D. W.; Holmes, L. S.; Baylis, S. A.; Rhodes, P.; Westmore, K.; Emson, P. C. and Moncada, S. (1995):** Roles of nitric oxide in tumor growth. *Proc. Natl. Acad. Sci*., 92(10): 4392-4396.
55. **Jin, Y.; Heck, D. E.; DeGeorge, G.; Tian, Y. and Laskin, J. D. (1996):** 5-Fluorouracil suppresses nitric oxide biosynthesis in colon carcinoma cells. *Cancer Res*., 56(9): 1978-1982.

56. **Jo, M.; Park, M. H.; Kollipara, P. S.; An, B. J.; Song, H. S.; Han, S. B.; Kim, J. H.; Song, M. J. and Hong, J. T. (2012):** Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. *Toxicol. Appl. Pharm*., 258(1): 72-81.
57. **Khalil, W. K.; Assaf, N.; ElShebiney, S. A. and Salem, N. A. (2015):** Neuroprotective effects of bee venom acupuncture therapy against rotenone-induced oxidative stress and apoptosis. *Neurochem. Int*. 80(15): 79-86.
58. **Kim, C. M. (2013):** Apitherapy–bee venom therapy. In Biotherapy-History, Principles and Practice. *Springer*, Netherlands, (pp. 77-112).
59. **Koçer, M. and Nazıroğlu, M. (2013):** Effects of 5-fluorouracil on oxidative stress and calcium levels in the blood of patients with newly diagnosed colorectal cancer. *Biol. Trace Elem. Res.*, 155(3): 327-332.
60. Kruiswijk, F.; Labuschagne, C. F. and Vousden, K. H. (2015): p53 in survival, death and metabolic health: a lifeguard with a licence to kill. *Nat. Rev. Mol. cell Boil*., 16(7): 393-405.
61. Lee, H. L.; Park, M. H.; Son, D. J.; Song, H. S.; Kim, J. H.; Ko, S. C.; Song, M. J.; Lee, W. H.; Yoon, J. H.; Ham, Y. W.; Han, S. B. and Hong, J. H. (2015): Anti-cancer effect of snake venom toxin through down regulation of AP-1 mediated PRDX6 expression. *Oncotarget*, 6(26): 22139-22151.
62. Lipps, B. V. (1999): Novel snake venom proteins cytolytic to cancer cells *in vitro* and *in vivo* systems. *J. Venom. Anim. Toxins*, 5(2): 172-183.
63. Lorenzo, L.; Chiara, P.; Antonio, C. and Calogero, F. (2012): Cancer pain management with venom of blue scorpion endemic in Cuba called *Rhopalurus junceus* "Escozul". *Open Cancer J*., 5(8):1-2.
64. Marnett, L. J. (1999): Lipid peroxidation—DNA damage by malondialdehyde. *Mutat. Res./Fund. Mol. Mech. Mut.*, 424(1): 83-95.
65. Masters J. R. (3rd Ed.). (2000): Animal cell culture: a practical approach (pp. 3-10). New York: Oxford University Press.
66. Moneim, A. E. A.; Ortiz, F.; Leonardo-Mendonca, R. C.; Vergano-Villodres, R.; Guerrero-Martínez, J. A.; López, L. C.; Acuna-Castroviejo, D. and Escames, G. (2015): Protective effects of melatonin against oxidative damage induced by Egyptian cobra (*Naja haje*) crude venom in rats. *Acta Trop*., 143(15): 58-65.
67. Nicotera, P.; Bonfoco, E. and Brüne, B. (1995): Mechanisms for nitric oxide-induced cell death: involvement of apoptosis. *Adv. Neuroimmunol*., 5(4): 411-420.
68. Nita, M. E.; Nagawa, H.; Tominaga, O.; Tsuno, N.; Fujii, S.; Sasaki, S.; Fu, C. G.; Takenoue, T.; Tsuruo, T. and Muto, T. (1998): 5-Fluorouracil induces apoptosis in human colon cancer cell lines with modulation of Bcl-2 family proteins. *Br. J. Cancer*, 78(8): 986-992.
69. Omran, M. A. A. (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: 66-86.
70. Ortiz, E.; Gurrola, G. B.; Schwartz, E. F. and Possani, L. D. (2015): Scorpion venom components as potential candidates for drug development. *Toxicon*, 93(1):125-135.
71. Park, M. H.; Choi, M. S.; Kwak, D. H.; Oh, K. W.; Yoon, D. Y.; Han, S. B.; Song, H. S.; Song, M. J. and Hong, J. T. (2011): Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-κB. *The Prostate*,71(8): 801-812.
72. Prado, M.; Solano-Trejos, G. and Lomonte, B. (2010): Acute physiopathological effects of honeybee (*Apis mellifera*) envenoming by subcutaneous route in a mouse model. *Toxicon*, 56(6): 1007-1017.
73. Shao, J.; Zhang, R.; Ge, X.; Yang, B. and Zhang, J. (2007): 45 Analgesic Peptides in *Buthus martensii Karsch*: A Traditional Chinese Animal Medicine. *Asian J. Tradit. Med*. 2 (2): 45-50.
74. Simon, H. U.; Haj-Yehia, A. and Levi-Schaffer, F. (2000): Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*, 5(5): 415-418.
75. Son, D. J.; Park, M. H.; Chae, S. J.; Moon, S. O.; Lee, J. W.; Song, H. S.; Moon, D. C.; Kang, S. S.; Kwon, Y. E. and Hong, J. T. (2007): Inhibitory effect of snake venom toxin from *Vipera lebetina turanica* on hormone-refractory human prostate cancer cell growth: induction of apoptosis through inactivation of nuclear factor κB. *Mol. Cancer Therap*., 6(2): 675-683.
76. Song, J. K.; Jo, M. R.; Park, M. H.; Song, H. S.; An, B. J.; Song, M. J., Han, S. B. and Hong, J. T. (2012): Cell growth inhibition and induction of apoptosis by snake venom toxin in ovarian cancer cell via inactivation of nuclear factor κB and signal transducer and activator of transcription 3. *Arch. Pharm. Res*., 35(5): 867-876.
77. Suhail, N.; Bilal, N.; Khan, H. Y.; Hasan, S.; Sharma, S.; Khan, F.; Mansoor, T.; FICS and Banu, N. (2012): Effect of vitamins C and E on antioxidant status of breast cancer patients undergoing chemotherapy. *J. Clin. Pharm. Ther*., 37(1): 22-26.
78. Swaim, M. W.; Chiang, H. S. and Huang, T. F. (1996): Characterisation of platelet aggregation induced by PC-3 human prostate adenocarcinoma cells and inhibited by venom peptides, trigramin and rhodostomin. *Eur. J. Cancer*, 32(4): 715-721.

79. Tu, W. C.; Wu, C. C.; Hsieh, H. L.; Chen, C. Y. and Hsu, S. L. (2008): Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. *Toxicon*, 52(2): 318-329.
80. Vyas, V. K.; Brahmbhatt, K.; Bhatt, H. and Parmar, U. (2013): Therapeutic potential of snake venom in cancer therapy: current perspectives. *Asian Pac. J. Trop. Biomed*., 3(2): 156-162.
81. Yeh, C. H.; Peng, H. C.; Yang, R. S. and Huang, T. F. (2001): Rhodostomin, a snake venom disintegrin, inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor growth by a selective αvβ3 blockade of endothelial cells. *Mol. Pharmacol.*, 59(5): 1333-1342.
82. Yin, X. Y.; Jiang, J. M.; Liu, J. Y. and Zhu, J. R. (2007): Effects of endogenous nitric oxide induced by 5-fluorouracil and L-Arg on liver carcinoma in nude mice. *World J. Gastroenterol.*, 13(46): 6249.
83. Zargan, J.; Sajad, M.; Umar, S.; Naime, M.; Ali, S. and Khan, H. A. (2011a): Scorpion (*Odontobuthus doriae*) venom induces apoptosis and inhibits DNA synthesis in human neuroblastoma cells. *Mol. Cell. Biochem*., 348(1): 173-181
84. Zargan, J.; Sajad, M.; Umar, S.; Naime, M.; Ali, S. and Khan, H. A. (2011c): Scorpion (*Androctonus crassicauda*) venom limits growth of transformed cells (SH-SY5Y and MCF-7) by cytotoxicity and cell cycle arrest. *Exp. Mol. Pathol*., 91(1): 447-454.
85. Zargan, J.; Umar, S.; Sajad, M.; Naime, M.; Ali, S. and Khan, H. A. (2011b): Scorpion venom (*Odontobuthus doriae*) induces apoptosis by depolarization of mitochondria and reduces S-phase population in human breast cancer cells (MCF-7). *Toxicol. In Vitro*, 25(8): 1748-1756.
86. Zhang, Y. Y.; Wu, L. C.; Wang, Z. P.; Wang, Z. X.; Jia, Q.; Jiang, G. S. and Zhang, W. D. (2009): Anti-proliferation effect of polypeptide extracted from scorpion venom on human prostate cancer cells *in vitro. J. Clin. Med. Res*., 1(1): 24-31.
87. Zheng, J.; Lee, H. L.; Ham, Y. W.; Song, H. S.; Song, M. J. and Hong, J. T. (2015): Anti-cancer effect of bee venom on colon cancer cell growth by activation of death receptors and inhibition of nuclear factor kappa B. *Oncotarget*, 6(42): 44437.
88. Zhou, J.; Qi, Y.; Diao, Q.; Wu, L.; Du, X.; Li, Y. and Sun, L. (2013): Cytotoxicity of melittin and apamin in human hepatic L02 and HepG2 cells *in vitro. Toxin Rev*., 32(4): 60-67.
89. Zhou, Q.; Sherwin, R. P.; Parrish, C.; Richters, V.; Groshen, S. G.; Tsao-Wei, D. and Markland, F. S. (2000): Contortrostatin, a dimericdisintegrin from *contortrix contortrix*, inhibits breast cancer progression. *Breast Cancer Res. Treat*., 61(3): 249-259.