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POTENTIAL ANTI CANCER ACTIVITY OF SNAKE VENOM, BEE VENOM AND THEIR COMPONENTS IN LIVER AND BREAST CARCINOMA

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ABSTRACT: The present study aimed to evaluate the antitumor efficacy of Snake venom (SV), bee venom (BV), combinations of their components and their apoptotic, cell death mechanisms in liver (HepG2) and breast (MCF7) cell lines. Cytotoxic effects of venoms, L-Amino acid oxidase (svLAAO), phospholipase A2(svPLA2), Melittin (MEL) were tested against HepG2 and MCF-7 cell lines and IC₅₀ was calculated. mRNA expression of p53, Bax and Bcl-2 was carried out. All the tested compounds had anti proliferative effects on the tumor cell lines with different potency. BV had a higher cytotoxicity against liver and breast cells (IC₅₀=1.26 and 2.85 µg/ml) than SV (IC₅₀=5.86, 13.05 µg/ml). The cytotoxicity of svLAAO was much higher than SV with IC₅₀ = 3.65 and 0.48 µg/ml. MEL showed a higher cytotoxic effect than BV against MCF7 cells (IC₅₀=1.14 µg/ml). MEL when combined with svPLA2 gave a synergistic effect on the expression of P53 and Bax in the two cancer cells. Venoms combination had the least synergistic effect relative to other combinations. From our results it could be concluded that SV, BV and their major components are promising anticancer agents for liver and breast carcinoma. SvLAAO is the most effective and safe compound (than its crude venom) to be used as antitumor agent or in combination with chemotherapeutic drug. MEL is a good candidate for liver and breast cancer treatment, it could be used in combined form with svPLA2 or svLAAO.

INTRODUCTION: Cancer is the major public burden in all developed and developing countries. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are projected to occur in year 2012 ¹. In all types of cancer, genetic alterations give rise to changes in expression, activation or localization of regulatory proteins in the cells, affecting the signaling pathways that alter their response to regulatory stimuli and allow the unrestricted cell growth.

Various therapies have been used for treating cancer such as chemotherapy, radiotherapy, immunotherapy and gene therapy ²; but still there is an urgent need of finding a better natural safe way to treat cancer with little effect on normal cells. Anticancer drug developments from natural biological resources are ventured throughout the world.

The biodiversity of venoms or toxins made it a unique tool from which new therapeutic agents may be developed. Several compounds from venomous animals, such as snakes, spiders, scorpions, caterpillars, bees, insects, wasps, centipedes, ants, toads, and frogs, have largely shown biotechnological or pharmacological applications ^{3, 4, 5, 6, 7}. This leads to search for cancer cure from natural products.

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Venom is a secretion of venomous animals synthesized in venom gland. It is a modified saliva containing a mixture of different bioactive proteins and polypeptides used by an animal for defense or to immobilize its prey⁸. Snake venoms consist of a mixture of proteins with or without catalytic activity such as phospholipase A₂ (PLA₂), proteases, hyaluronidases, L-amino acid oxidases (LAAOs), acetylcholinesterases, growth factors, protein C activators, lectins, and von Wille brand factor-binding proteins; peptides mainly comprising bradykinin potentiators and disintegrins; also it contains low molecular weight organic compounds (carbohydrates, serotonin, histamine, citrate, and nucleosides) and inorganic ions such as (calcium, cobalt, magnesium, copper, iron, and potassium) as well as enzymatic inhibitors⁹.

Not only the venom of every snake is different but a subtle difference exists between different species, between juveniles and adults, even among the snake of same species but of different geographical regions. Approximately 90-95% of venom's dry weight is composed of proteins that may be toxic or non-toxic¹⁰. The ability of snake venoms to act upon tumor cells has been known for a long time. The first reported studies on using snake venom against tumor cells were related to the defibrination process¹¹.

L-amino acid oxidase (LAAO) constitutes 1-9% of the total venom protein and is responsible for the light yellowish color of the venom¹² it has been reported to exhibit a wide range of pharmacological activities including anti-proliferative¹³ and anti-bacterial activities¹⁴. It can induce apoptosis in mammalian endothelial cells due to the production of high concentration of hydrogen peroxide which kills the cells¹². Phospholipase A₂ (PLA₂) plays an important role in many biological events such as cell signaling and cell growth, generation of pro-inflammatory lipid mediators such as prostaglandin, and leukotrienes¹⁵.

Snake venoms are the richest sources of PLA₂ which display a variety of relevant toxic actions such as neurotoxicity, cytotoxicity, cardiotoxicity, hypotensive and proinflammatory effects^{16,17}.

Bee venom (BV) contains a variety of different peptides, including melittin, phospholipase A₂, apamin, adolapin and mast cell-degranulating peptide (MCDP); it also contains non-peptide components including lipids, carbohydrates and free amino acids, all having many cellular activities¹⁸. BV has been used as a traditional medicine to treat back pain, rheumatism, and skin diseases due to its antibacterial, antiviral, and anti-inflammatory effects¹⁹. Several studies have demonstrated that bee venom either or melittin have anti-proliferative effects on various cancer cells such as prostate, liver, breast, cervical and renal cancer cells through intrinsic or extrinsic apoptosis^{20, 21, 22, 23}.

Melittin (MEL) is a major peptide constituent of bee venom that has been proposed as one of the upcoming possibilities for anticancer therapy. Recent reports point to several mechanisms of MEL cytotoxicity in different types of cancer cells such as cell cycle alterations, effect on proliferation and/or growth inhibition, and induction of apoptotic and necrotic cell death through several cancer cell death mechanisms, including the activation of caspases and matrix metalloproteinases²⁴.

However, little is known about the anticancer activity of crude SV, BV and their venomous major components against liver and breast cancers which are the most spread types worldwide. Also there is no information about the synergistic or antagonistic anticancer effects of the combined treatment with venoms and venomous materials. So the current study aims to evaluate the anticancer activities of crude BV, SV, their major components (LAAO, PLA₂, MEL) as well as their combinations against liver and breast cancer cells and also the effects of these tested compounds on the expression of the apoptotic genes and on the cell cycle of the tested tumor cells.

MATERIALS AND METHODS:

Materials:

Chemicals:

RPMI 1640 medium with L- glutamine (Biowhittaker - Belgium), Fetal bovine serum (FBS) (GIBCO - USA), dimethyl sulfoxide (DMSO) solvent and other cell culture materials were purchased from Fisher Scientific Cell Culture

(Houston, TX, USA). Other reagents were of the highest analytical grade available.

Culture cells and venoms:

Human breast adenocarcinoma cells (MCF7 - ATCC number: HTB-22), human hepatocellular carcinoma cells (HepG2 ATCC number HB-8065), non tumorigenic normal human lung fibroblast (MRC-5 cells – ATCC No. CCI-171), snake venom: Naja haje (Egyptian cobra) and bee venom from honey bee *Apis mellifera* were supplied from Vaccera sera plant (Egypt).

L-Amino acid oxidase from snake venom (svLAAO) , svPLA2 from snake venom 1,500 units/ mg protein (lyophilized powder) and Melittin (MEL) from honey bee venom: *Apis mellifera*, Purity 96.50% were purchased from Sigma company(Sigma-Sain Diago- USA).

RNA and apoptotic genes:

Bioopure RNA isolation reagent were purchased from Bio Scientific Group Austin, USA. Maxima first strand cDNA synthesis Kit for RT-q PCR and Primer sequences of P53, Bax and Bcl-2 genes were supplied from Thermo Scientific USA.

Cytotoxicity assays:

Cytotoxic effects of venoms(Snake and Bee), L-Amino acid oxidase(LAAO), PLA2 and MEL were tested against MCF- 7, HepG2 and MRC-5 in vitro cell lines by the MTT assay as previously described by Alley et al., 1988²⁵. Negative cell control was included. IC50 was calculated by using Masterplex 2010 hitachia (GIRSS). Data were presented as IC50 of the tested compounds compared to that of normal cell control MRC-5. Five combinations of venoms and venomous components were prepared from the resulted IC50 value of each compound for evaluation of their apoptotic effects on the tested tumor cell lines. These combinations are: BV + SV (combination 1),

LAAO+PLA2(combination2),LAAO+MEL(combination3),PLA2+MEL(combination4),MEL+LAAO+PLA2(combination5).

Apoptotic evaluation:

RNA was extracted from 24 hr treated cells with venoms, LAAO PLA2, MEL, the 5 combinations

mentioned above, treated and untreated control cells by using RNA isolation kit according to manufacturer's protocol. Extracted RNA was reverse transcribed to cDNA using Maxima first strand cDNA synthesis Kit. The mRNA expression of pro-apoptotic genes (P53 and Bax) and anti-apoptotic gene (Bcl-2) was carried out using the newly synthesized cDNA as template for PCR. Semi-quantitative RT-PCR was carried out according to²⁶. Using the specific primer for each gene (in a concentration of 10pmole/ul for each primer) and housekeeping gene (GAPDH).

Gel electrophoresis for PCR products (10µl) was carried out on 1.5% agarose gel using DNA ladder 1.5 KB and visualized using UV transilluminator after staining with ethidium bromide followed by densitometric analysis of bands intensities which expressed as relative absorbance units. Data representing mRNA expression levels of p53, Bax and Bcl-2 were calculated as band intensities compared to GAPDH by using alpha gel documentation system.

Cell cycle analysis:

Cell cycle analysis is carried out according to the method of (Mansouri M et al 2014)Each cell line type (MCF7 , HepG2 and MRC5) was seeded into 96-well plates at a density of 6×10^5 cells/well for 24 h. Then, the cells were treated with 100 mM SV, BV , LAAO and MEL for 24 h. Approximately 2.5×10^5 cells were harvested, washed twice using ice-cold PBS, and re suspended in 200 ml of cold PBS (phosphate –buffered saline). For rapid cell fixation and dispersion, the cell suspensions were added drop wise to 1 ml of cold 70% ethanol and incubated on ice for 45 min.

After centrifugation, the cells were resuspended in 1ml of propidium iodide (PI) master mix (PBS containing 100 mg/ml RNase A and 40 mg/ml PI) and incubated in the dark at 37 °C for 30 min. The various cell cycle phases were monitored using a FACSCalibur_ flow -cytometer (BD Biosciences, San Jose, CA). Cells were excited at 488 nm with an argon laser, and the emission from ten thousand cells was recorded using a 580 nm band-pass filter (FL2-H)²⁷. The obtained cell cycle profiles were analyzed using CellQuest version 3.2 and Win MDI version 2.8 softwares.

RESULTS:

1-Cytotoxic effects of crude Snake and Bee venoms against MCF7 and HepG2 cells: The tested concentration is 100ug/ml**

Both venoms have an anti proliferative effects on HepG2 and MCF7 cell lines. Bee venom shows a significant high cytotoxic activity against both types of tumor cells exceeds that to normal cell control MRC-5 with 50% inhibition concentrations (IC50) of 1.26 and 2.85µg /ml respectively compared to MRC-5 (77.7µg /ml) while snake venom exerts a different effect; it affect the proliferation of the liver cancer cells (IC50=5.86 µg /ml) more than the breast cells IC50 = 13.05µg /ml compared to MRC-5 cells (IC50=26.118µg /ml), (Table 1, Fig. 1, 2).

1-A Cytotoxic effects of LAAO, PLA2:**

LAAO as one of the components of snake venom shows more potent inhibitory activity than the

crude snake venom on both HepG2 cells (IC50=3.65 µg /ml) and MCF7(IC50 =0.487 µg /ml) compared to MRC-5 normal cells (IC50=140.7µg /ml) in the tested concentration of 100 µg /ml while the opposite is shown with PLA2 since it gives a much lower cytotoxic effect than the crude venom on liver (IC50 =53.6µg /ml) and breast (27.6 µg /ml) cancer cells compared to normal cells (IC50= 8.46µg /ml) in the tested concentration of 20ug/ml (Table 1, Fig.2-4).

1-B Cytotoxic effect of Melittin: (tested concentration is 20µg/ml)**

Melittin as the main component of Bee venom shows high activity on MCF7 (IC50 =1.14µg /ml) than the crude bee venom in contrast to its effect on HepG2 cells (IC50=6.12µg /ml) , on the other hand the IC50 of MEL on MRC-5 cells (IC50=8.1µg /ml) is higher that of the crude venom (Table 1 and Fig. 3, 4) .

TABLE 1: IC50 VALUES OF VENOMS AND VENOMOUS COMPONENTS IN MCF7, HEPG2 AND MRC5 TREATED CELL LINES.

IC50 Cell line	Snake Venom (Copra)	svLAA	svPLA2	Bee Venom	Melittin
MCF7	13.0572	0.4875	53.6	2.85	1.14
Hep G2	5.86	3.65	27.6	1.26	6.12
MRC5	26.11	140.7	84.6	77.7	8.1

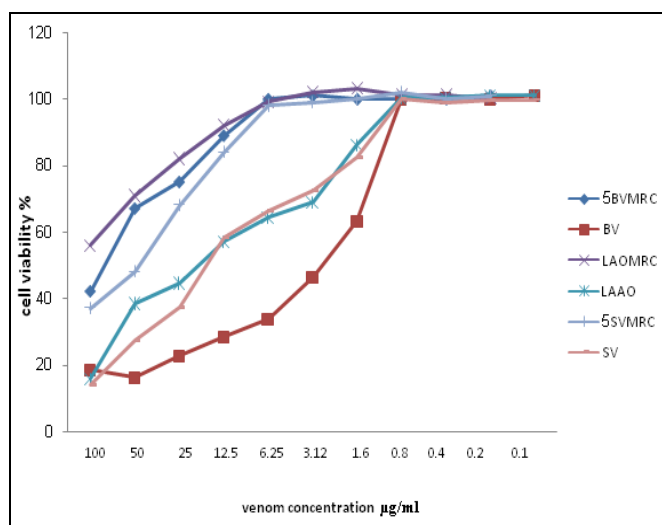


FIG.1: EFFECT OF SV, BV, LAAO ON CELL VIABILITY OF MCF-7 AND MRC5 CELL LINES. RESULTS ARE EXPRESSED AS PERCENTAGE OF VIABLE CELLS. CELLS WERE SEEDED IN 96 -WELL TISSUE CULTURE PLATES AND TREATED AFTER 24H WITH BV, SV, AND LAAO. CELLS WERE INCUBATED FOR 72H, PERCENT OF CELL VIABILITY WAS CALCULATED IN RELATION TO THE UNTREATED CONTROL. MTT COLORIMETRIC ASSAY METHOD WAS USED FOR DETERMINATION OF CELL VIABILITY.

Apoptotic effects of crude venoms and their components:

On the molecular level the effects of the tested compounds on mRNA expression of proapoptotic (p53, Bax) and anti apoptotic (Bcl2) genes in MCF7 and HepG2 cancer cells were determined semiquantitatively by RT-PCR followed by densitometrical analysis of the resulted gel electrophoresis bands.

All the tested compounds increase the expression of p53 and decrease that of Bcl2 in the treated cells with different potency (Fig. 5, 7, Table 2). Concerning the expression of Bax, variable results have been observed. MEL does not increase the expression of Bax (negative effect) in the two treated cells, while SV has a positive effect (increase in expression) on Bax expression in MCF7 and a negative effect in HepG2 cells .The remain tested compounds have positive activity on the two treated cancer cells (Fig.6, Table 2).

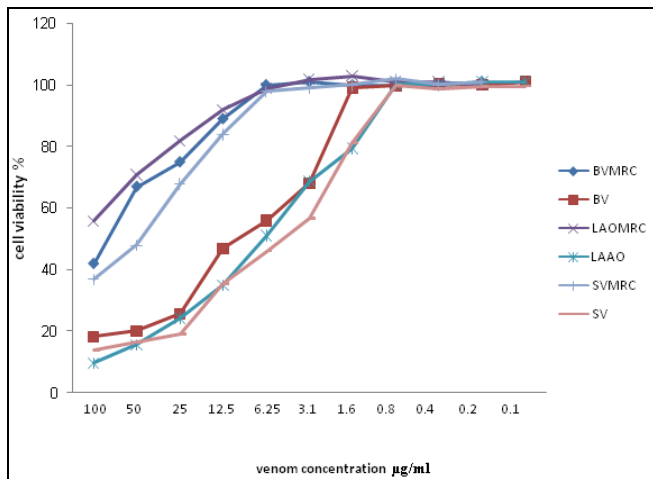


FIG. 2: EFFECT OF SV, BV, LAAO ON CELL VIABILITY OF HEPG2 AND MRC5 CELL LINES.

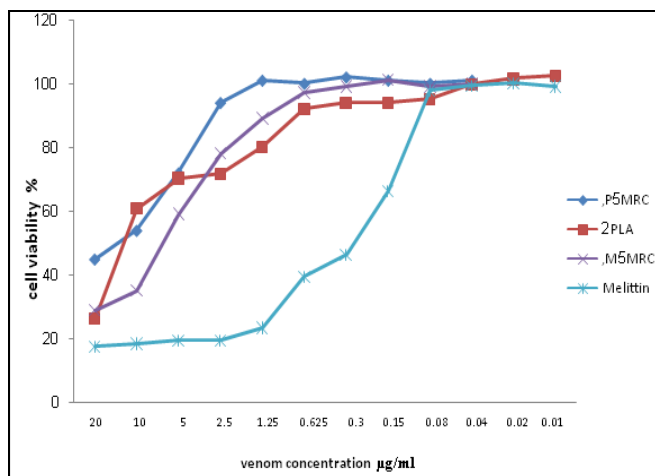


FIG.3: EFFECT OF MEL AND PLA2 ON CELL VIABILITY OF MCF7 AND MRC5 CELL LINES. RESULTS ARE EXPRESSED AS PERCENTAGE OF VIABLE CELLS. CELLS WERE SEED IN 96 -WELL TISSUE CULTURE PLATES AND TREATED AFTER 24H WITH BV, SV, AND LAAO. CELLS WERE INCUBATED FOR 72H, PERCENT OF CELL VIABILITY WAS CALCULATED IN RELATION TO THE UNTREATED CONTROL. MTT COLORIMETRIC ASSAY METHOD WAS USED FOR DETERMINATION OF CELL VIABILITY.

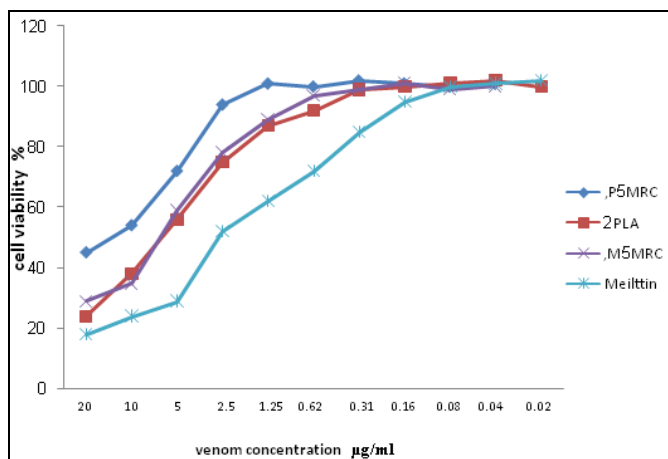


FIG.4: EFFECT OF MEL AND PLA2 ON CELL VIABILITY OF HEPG2 AND MRC5 CELL LINES.

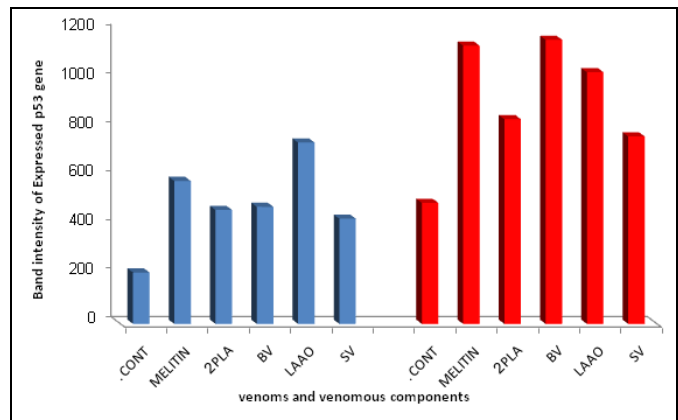


FIG.5: EFFECT OF VENOMS AND VENOMOUS COMPONENTS ON THE EXPRESSION OF P53 GENE IN LIVER (A) AND BREAST (B) CANCER CELL LINES. RNA WAS EXTRACTED FROM 24 HR TREATED CELLS, TREATED AND UNTREATED CONTROL CELLS .THE MRNA EXPRESSION OF P53 GENE WAS CARRIED BY SEMI-QUANTITATIVE RT-PCR METHOD FOLLOWED BY GEL ELECTROPHORESIS FOR PCR PRODUCTS AND DENSITOMETRIC ANALYSIS OF BANDS INTENSITIES WHICH EXPRESSED AS RELATIVE ABSORBANCE UNITS. DATA REPRESENTING MRNA EXPRESSION LEVELS OF P53 WAS CALCULATED AS BAND INTENSITIES COMPARED TO GAPDH BY USING ALPHA GEL DOCUMENTATION SYSTEM.

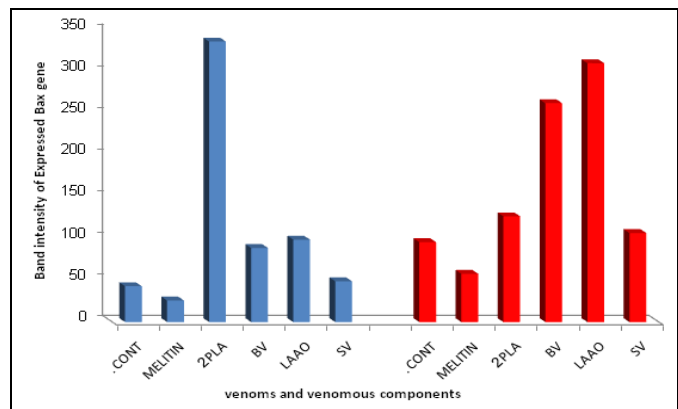


FIG. 6: EFFECT OF VENOMS AND VENOMOUS COMPONENTS ON THE UP-EXPRESSION OF BAX GENE IN LIVER (A) AND BREAST (B) CANCER CELL LINES. DATA REPRESENTING MRNA EXPRESSION LEVELS OF BAX WAS CALCULATED AS BAND INTENSITIES COMPARED TO GAPDH BY USING ALPHA GEL DOCUMENTATION SYSTEM.

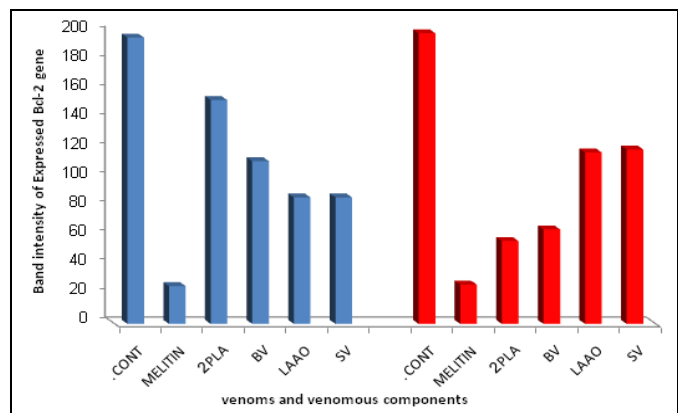


FIG.7: EFFECT OF VENOMS AND VENOMOUS COMPONENTS ON THE DOWN-EXPRESSION OF BCL-2 GENE IN LIVER (A) AND BREAST (B) CANCER CELLS. DATA REPRESENTING MRNA EXPRESSION LEVELS OF BCL-2 WAS CALCULATED AS BAND INTENSITIES COMPARED TO GAPDH BY USING ALPHA GEL DOCUMENTATION SYSTEM.

TABLE 2: MRNA EXPRESSION LEVELS OF APOPTOTIC GENES POST 24H TREATMENT OF LIVER AND BREAST CANCER CELLS WITH SV, BV, LAAO, PLA2, MEL AND THEIR COMBINATIONS. L+P=COMBINATION OF LAAO WITH PLA2, L+M= LAAO+MEL, P+M= PLA2+MEL, M+L+P= MEL +LAAO+PLA2

Hep G2 cell lines						MCF-7 cell lines				
Gene	S.V	B.V	LAAo	PLA2	MEL	S.V	B.V	LAAo	PLA2	MEL
P53	++	+++	++++	++	+++	++	+++	+++	++	+++
Bax	-	++	++	+++	-	+	++	+++	+	-
Bcl-2	+++	++	+++	+	++++	++	+++	++	+++	++++
Gene/combinations	S.V + B.V	L+P	L+M	P+M	M+L+P	S.V + B.V	L+P	L+M	P+M	M+L+P
P53	+	++	++++	++++	++	+	++	+++	++++	+
Bax	+	+++	++++	++++	+	++	+++	++++	++++	+++
Bcl-2	++	++++	+	+++	++++	+++	+++	++	++	+

Synergistic and/or antagonistic apoptotic activity of combined treatment with venoms and venomous components:

Five combinations were tested for assessment of their synergistic and or antagonistic regulatory effects on the expression of apoptotic genes. Combination 2 (LAAO+PLA2) shows equal synergistic activity on the expression of pro and anti apoptotic genes in liver and breast cancer cells; while addition of MEL to LAAO or PLA2 (combinations 3and 4) gives different effects.

MEL causes an increase of the activity of PLA2 (combination 4) and gives the highest synergistic activity on the expression of p53, Bax genes in breast and liver cancer cells as well as BCl2 in liver cancer cells, while LAAO efficiency does not affected by combining with MEL .

Combination 5 (MEL + LAAO + PLA2) shows high synergistic effect on the down expression of Bcl2 in liver cancer cells with contrast effect on breast cancer cells (lowest synergistic effect).

The crude venoms combination shows variable effects in liver cells, it gives the least synergistic effect on p53 and Bcl2 than the effect of individual venom either SV or BV, but the contrast is shown with the up regulation of Bax since addition of BV increases the apoptotic effect of SV and leads to increase of Bax expression. **Table 2 and Fig. 8, 9, 10, 12** show the different potential apoptotic effects of the tested combinations in the treated cancer cells.

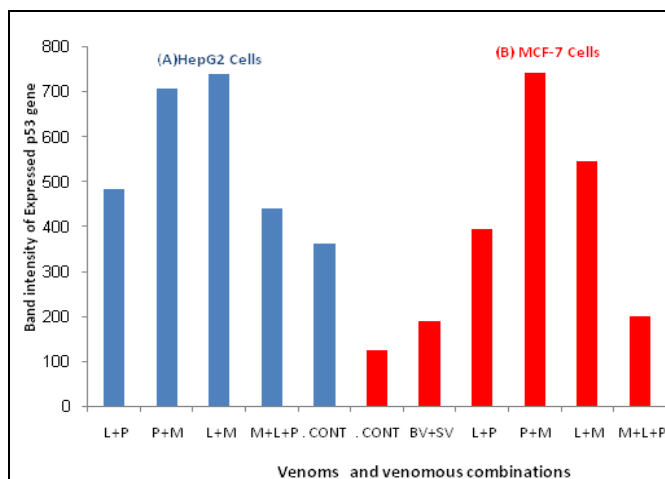


FIG. 8: EFFECT OF VENOMS AND VENOMOUS COMPONENTS COMBINATIONS ON THE EXPRESSION OF p53 GENE IN (A) LIVER AND (B) BREAST CANCER CELLS. CONT.: control (untreated cells), BV+SV=Bee Venom and Snake Venom combination L+p=svLAAO+svPLA2 combination, P+M=svPLA2+MEL combination, L+M=svLAAO +MEL combination, M+L+P= MELL +svLAAO + svPLA2 combination. Data representing mRNA expression levels of P53 was calculated as band intensities compared to GAPDH by using alpha gel documentation system.

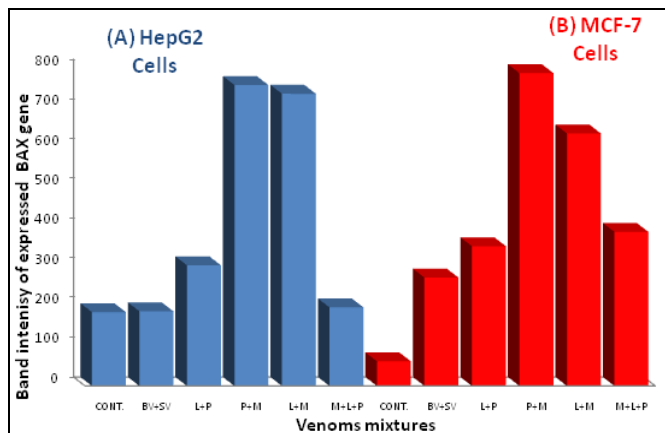


FIG.9: EFFECT OF VENOMS COMBINATIONS ON THE UP-EXPRESSION OF BAX GENE LIVER IN (A) AND BREAST (B) CANCER CELLS .

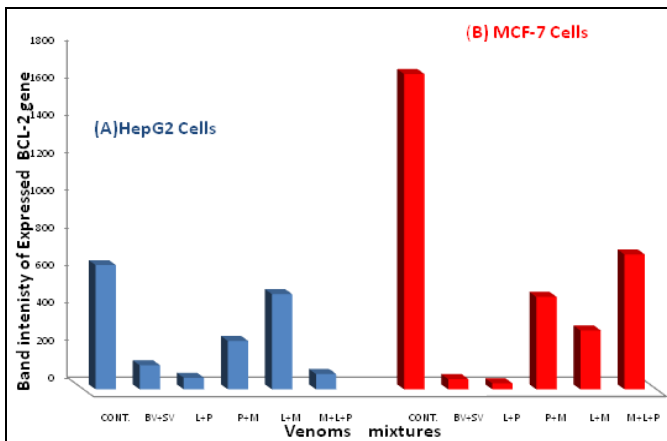


FIG. 10: EFFECT OF VENOMS COMBINATIONS ON THE EXPRESSION OF BCL-2 GENE IN LIVER (A) AND BREAST (B) CANCER CELL LINES.

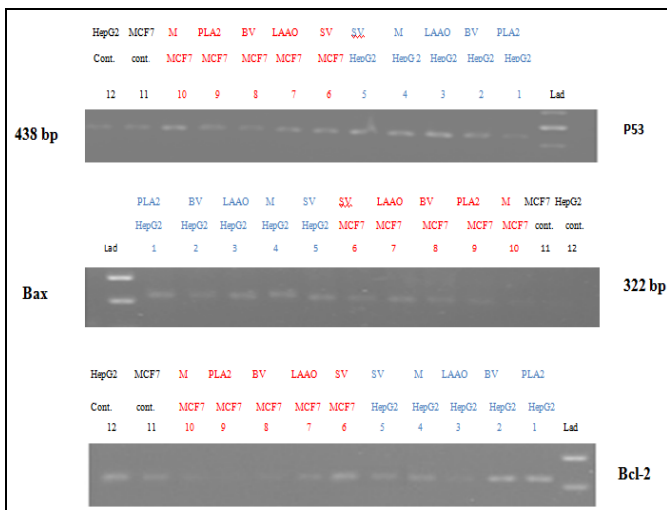


FIG. 11: MRNA EXPRESSION OF APOPTOSIS RELATED GENES IN HEPG2 AND MCF7 CELLS POST TREATMENT WITH SV, BV, LAAO, PLA2 AND MELITTINE (M) HepG2: lanes 1-5, MCF7 (lanes 6-10), Lanes 11, 12: untreated control cells of HepG2 and MCF7 cells. Lad: DNA marker

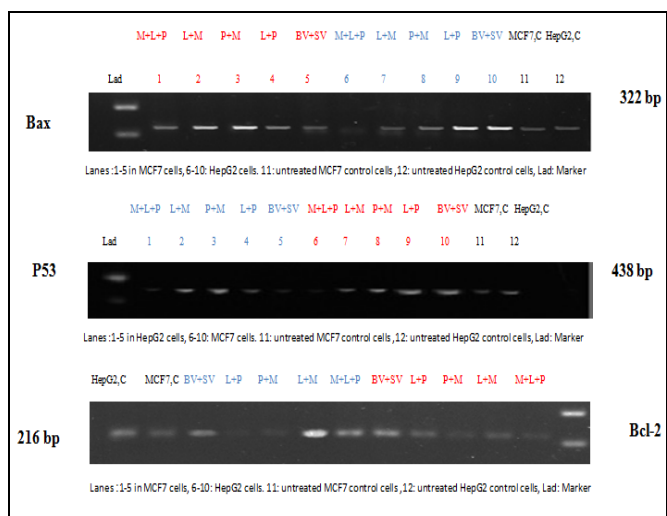


FIG. 12: MRNA EXPRESSION OF APOPTOSIS RELATED GENES IN HEPG2AND MCF7) CELLS POST TREATMENT WITH DIFFERENT VENOMS AND VENOMOUS COMPONENTS COMBINATIONS.

Cell cycle arrested at theG0/G1 phase by tested venoms

All the tested venoms and venomous components arrest the treated cells at G0/G1 phase. Fig.13 demonstrate the different percentage of cell cycle arrest at G0/G1 phase of 24h treated cells with venoms and venomous components.

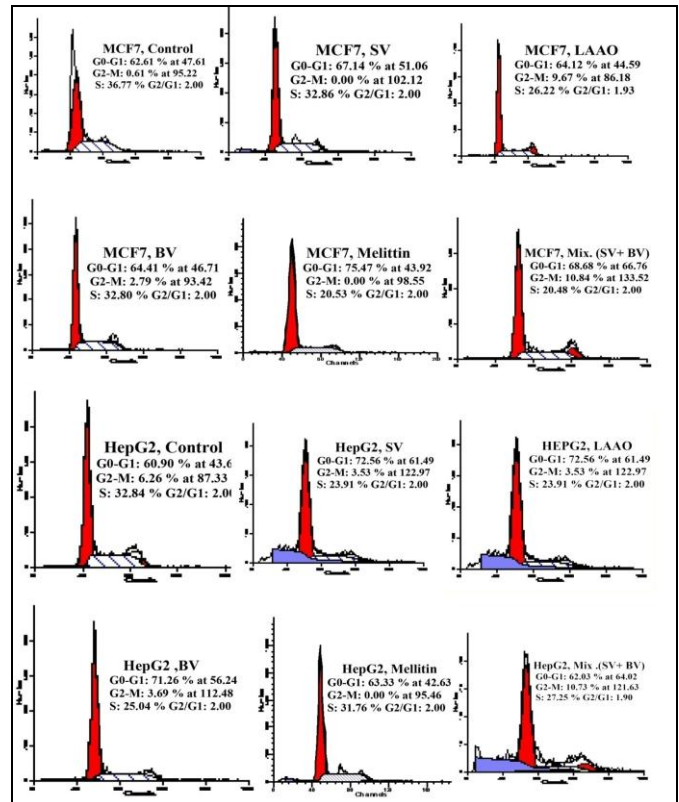


FIG.13: FLOWCYTOMETRIC ANALYSIS POST 24 H TREATMENT OF HEPG2 AND MCF7 WITH VENOMS, VENOMOUS COMPONENTS AND THEIR COMBINATIONS

DISCUSSION: Although the use of chemotherapeutics in cancer therapy remains the predominant option for clinical control; it provides inadequate effect in addition to its effect on the normal cells as well as on cancer cells; also one of the major problems of chemotherapy is the resistance developed after initial treatment.

This has led to the increased demand of using anticancer drugs developed from natural resources. The biodiversity of venoms and toxins makes them a unique source from which novel therapeutics may be developed. Snake and bee venoms reported to have a potential cytotoxic effects on tumor cells [28]. However, little is known about their cytotoxicity as crude venoms compared with their major components (LAAO, PLA2, MEL) in breast

and liver carcinoma which are considered to be the most spread cancer types worldwide. In the current study the anti proliferative effects of SV (copra), BV, svLAAO svPLA2 and MEL have been shown on liver and breast cancer cells. Snake venom contains cystatin (a member of cysteine protease family inhibitors) which has been reported to play an important role in tumor invasion and metastasis. In a study carried out on MHCC97H (liver cancer) cells, sv-cystatin has shown inhibition of tumor cell invasion and metastasis through the reduction of the proteinases activity and epithelial-mesenchymal transition (EMT)²⁹.

On the other hand the cytotoxins from *some snake species* (*N. haje*, *N. oxiana*, and *N. kaouthia*) can readily penetrate into living cancer cells as human lung adenocarcinoma A549, promyelocytic leukemia HL60 and markedly accumulate in lysosomes (which are the main targets of cytotoxins) and cause lysosomal leakage and plasma membrane injury³⁰. However there are many toxins from snake venom formulated into drugs for the treatment of cancer, hypertension and thrombosis^{31, 32, 33, 34}; the potency of venom and its effect on human depend on the type and amount of venom injected and the site where it is deposited³⁵.

The current study shows the potent efficacy of svLAAO on breast and liver tumor cells (more than that of the crude SV) which is in agreement with different studies that isolate LAAO and reported its cytotoxicity in stomach, colorectal, adenocarcinoma, human fibroblast cell lines³⁶, MCF7 and CACO-2 cancer cell lines³⁷, and on breast and lung tumorigenic cells³⁸.

The present study supports a large number of studies that demonstrated the antitumor properties of BV and in particular of its major constituent MEL. Several mechanisms of BV cytotoxicity in different types of cancer cells have been reported such as cell cycle alterations, effect on proliferation and/or growth inhibition, and induction of apoptosis or necrosis^{39, 40, 41, 42, 43, 44, 45}. Also, the anti proliferative effects of BV has been recently demonstrated on the cervix C33A cancer cells⁴⁶ and lung cancer cells^{47, 48}. On the other hand the anticancer activity of MEL has been approved since 2008⁴⁹ on HCC cells in nude mouse models

.MEL shares its amphipathic properties as one of the peptides isolated from BV that are characterized by their capacity to disturb tumor cell membrane bilayer integrity, either by creation of defects, disruption, or through pore formation with opening in the lipid bilayer leads to the collapse of transmembrane electrochemical gradients. In contrast to normal cells with low membrane potential, tumor cell membrane has a large membrane potential^{50, 42, 51} therefore many lytic peptides selectively disrupt the membrane structure of tumor cells to a greater extent than the membrane of normal cells⁵¹.

Although MEL shows a potent cytotoxicity against tumor cells in the present study, it has also a remarked toxicity to normal cells exceeds that of all tested components; this result is in agreement with (Soman et al., 2009) and (Pan et al., 2011) in that MEL is also toxic to normal cells and its therapeutic potential cannot be achieved without a proper delivery vehicle^{52, 53}. On the other hand it was approved that MEL does not prevent the growth of normal cells at a concentration which prevents the proliferation of tumor cells⁵⁴ therefore it could play a useful role in the anticancer treatment⁵¹.

The regulation of apoptosis in normal and malignant cells has become an area of extensive study in cancer research⁵⁵ since the apoptotic process is involved in the growth and inhibition of the tumor cells. In the present study we examine the apoptotic regulatory effect of the tested venoms and their components as well as their combinations in liver and breast cancer cells through the increase in expression intensity of P53, Bax and decrease in that of Bcl2 resembling that approved by different studies on the relation between venoms and their cancer cell death by apoptotic process. The effect of BV on human lung cancer cells^{47, 48} and cervix cancer C33A cells⁴⁶ was reported through enhancement of death receptor (DR) expressions with increase in expression of pro-apoptotic proteins, up-regulated Bax and decrease in Bcl-2.

NF- κ B. On the other hand similar studies reported the inhibitory effect of SV toxin on the growth of colorectal cancer cells through induction of intrinsic or extrinsic apoptosis⁵⁶ and DR mediated

apoptosis⁵⁷. Also the apoptotic cell death of MEL has been reported against different in vitro tumor cell lines as in human hepatoma and glioma cell lines^{41, 58}, hepatocellular carcinoma and osteosarcoma cell lines^{59, 60, 61}. Furthermore MEL prevents liver cancer metastasis through inhibition of the Rac1- dependent pathway⁴⁹; also it induces apoptosis in leukemic cells through the up-regulation of Bax and caspase-3 activation, down-regulation of Bcl-2 and the inhibitor of apoptosis protein (IAP) family members⁶².

The apoptotic effect of LAAO also reported in human prostate adenocarcinoma (PC-3) model through increase in caspase-3/7 cleavage³⁸.

Few studies reported the possible anti tumor effects of either venom and venom derivative combinations or their combinations with chemotherapeutic drugs. To our knowledge the current study is the first one which investigate the possibility of using the tested venoms and /or their components in combined form as anticancer agents for liver and breast cancer. Lipps BV 1999, reported the synergetic activity of SV proteins atroporin and kaotree against cancer cells⁶³. On the other hand the most important benefits of using the combination of a chemotherapeutic drug with venom or venomous component is to reduce the required therapeutic dose of either one , minimize their side effects and also decrease the cancer cell resistance ; this information was recently confirmed by (Gajskia G et al.2013) in his study on the synergistic effect of using BV in combination with cisplatin on human cervical and laryngeal carcinoma cells which could be useful in minimizing the cisplatin concentration during chemotherapy, consequently reducing and/or postponing the development of cisplatin resistance⁶⁴.

The current study shows the synergistic apoptotic effect of using PLA2 in combination with MEL in liver and breast cancer cells which may be explained by the proposal of (Holle et al., 2003 and Moon et al., 2006) that BV and its peptide components are related to the activation of PLA2, caspase and matrix metalloproteinase that destroy tumor cells^{50,42}; also (Son et al., 2007) reported that MEL increases the activity of PLA2 on rat

oncogen transformed cells resulting in their selective destruction⁵¹. However, MEL is considered to be a very attractive candidate for cancer chemotherapy because cancer cells are less likely to develop resistance to a membrane pore-former so the combination of a chemotherapeutic drug together with MEL could be useful synergistic therapy⁶⁵. ON the other hand MEL could be used in hepatocellular carcinoma since it can synergies with TNF-related apoptosis-inducing ligand (TRAIL) in the induction of hcc apoptosis by activating the TAK1-JNK/p38 pathways⁶⁶.

Regarding the use of crude venoms combination, the present study shows that SV and BV combination gives the least pro-apoptotic regulatory effect on the expression of p53 and Bax genes in the two tested cancer cells which may be due to certain antagonism/ interaction between venoms components when used in combined form.

Conclusions and future suggestions:

Finally it can be concluded that: SV, BV and their major components are promising anticancer agents for liver and breast carcinoma. sv LAAO is the most effective and safe compound (than its crude venom) to be used as antitumor agent or in combination with chemotherapeutic drug. On the other hand MEL is a good candidate for liver and breast cancer treatment, it could be used in combined form with svPLA2 or svLAAO.

It can be recommended that more *in vitro* animal studies are required to identify the suitable therapeutic safety dose of these venomous compounds or combinations and their possible side effects on normal cells., also several trials are recommended to deliver these compounds in a suitable pharmaceutical form (as nanoparticles) which could minimize their toxicity ,target the tumor cells and facilitate their separate use or in combination with other chemotherapeutic agents.

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