Research Paper

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In vitro Anticancer Effect of Scorpion *Leiurus quinquestriatus* and Egyptian Cobra Venom on Human Breast and Prostate Cancer Cell Lines

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Venoms and toxins from some snakes are known to influence the growth of cancer cells. In this study we determine the potential efficacy, the cytotoxic and antitumor effects of scorpion *L. quinquestriatus* and snake *Naja haje* venoms, two potent toxins, on two established human breast and four prostate cancer cell lines. The effect of both venoms on cancer cells survival and the 50% lethal concentration (LC_{50}) was determined during 24 h incubation with three different concentrations. Morphological and pathological alterations to all treated cell lines were also monitored. Snake venom is more potent as antitumor agent than scorpion venom for both breast and prostate cancer cell lines. The results showed high selectivity of scorpion toxins for the breast cancer T47D cell line. LC_{50} values of cobra venom in the two breast cancer cell lines, were 18 µg/ml in T47D cells and 63 µg/ml in MDAMB-468 cells. On the other hand, the range of the LC_{50} values in case of prostate cancer cell lines were between 38 and 61 µg/ml. Percentage of cell death was time and dose-dependent in most cell lines. Cobra venom induced significant cell death of breast cancer cell line T47D 98% and MDAMB-468 75% after 24 h incubation with 100 µg/ml. Also, the highest snake venom dose killed 98, 94 and 84% of prostate carcinoma DU145, PC-3 and TSUpr1, respectively. Morphologically, dying cells showed fragmentation, condensation of their contents concomitant with shrinkage and appearance of vacuoles between adherent cells. Cells were swollen before they destruct and cellular debris was observed in the media with floating dead cells. In conclusion, results provides a rational for *in vivo* studies to determine whether scorpion *L. quinquestriatus* and Egyptian cobra venoms will provide effective therapy for breast and prostate cancer especially in the cases of metastasis.

Key words: Scorpion venom, cobra venom, cytotoxicity, cancer

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Introduction

Cancer is a group of neoplastic diseases that occur in humans of all age groups and races as well as in all animal species. Tumor cells are characterized by uncontrolled growth, invasion to surrounding tissues and metastatic spread to distant sites. Mortality from cancer is often due to metastasis since surgical removal of tumors can enhance and prolong survival (Gerald, 1999). The breast is the most common site of cancer in women and breast cancer is second only to lung cancer as a cause of death from cancer among women (Awad ***et al.***, 2000). In UK, the predicted breast cancer incidence in year 2020 is 39 000 and the predicted cancer mortality from this disease is 19 000, which about 50% of the diseased women (Sikora, 1999a). In adult males, prostate cancer is the most common neoplasm and the most common cause of death due to cancer (Kumi-Diaka, 2002). By the year 2020, the predicted prostate cancer cases in UK is 22 5000 and the predicted number of deaths is about 15 500, that represent 68.8% mortality.

Drug research may provide the ultimate cure for cancer, although many of the currently available agents fall short of this goal. A major effort to develop anticancer drug through both empirical screening and rational design of new compounds has now been under way for 4 decades (Durand-Fontanier ***et al.***, 1999; Awad ***et al.***, 2000; Exermann ***et al.***, 2002). Recent advances in this field have included the synthesis of peptides and proteins with the recombinant DNA technique and monoclonal antibodies. Ideal anticancer drugs would eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents can meet this criterion and clinical use of these drugs involves a weighing of benefits against toxicity in a research for a favourable therapeutic index.

Venomous creatures have always had a peculiar fascination in both real-life and fiction and the nature of their venoms, how they act and how they can be neutralized are intrinsically important fields of research. In recent years, however, interest has extended beyond this into the development of new protein and peptide substances of therapeutic importance. De Carvalho ***et al.*** (2001) suggested that the lectins which are polyvalent carbohydrate-binding proteins of non-immune origin that have been isolated and characterized from the venom of the snake ***Bothrops jararacussu*** may serve as an interesting tool for combating tumor progression by inhibiting tumor cell, human metastatic breast cancer (MDA-MB-435) and human ovarian carcinoma (OVCAR-5) cell lines and bovine brain endothelial cell growth. Some other investigators (Chiang ***et al.***, 1995; Costa ***et al.***, 1998; Maristela ***et al.***, 1999; Zhou ***et al.***, 2000) reported the anticancer effects of lectins and other novel toxins isolated from snake venoms against some types of tumors.

Scorpion ***Leiurus quinquestriatus*** venom contains a number of polypeptide toxins that specifically block or alter gating properties of ion channels. Their toxicity to mammals is due to the large single-chain proteins (60-70 amino acids) with four disulfide bridges that mainly affect sodium channels (Moskowitz ***et al.***, 1998). Venom from the scorpion ***L. quinquestriatus*** is about

the most lethal of the scorpion venoms with an LD_{50} of 0.25 mg/kg of mouse (Gwee ***et al.***, 1996). Despite the diverse biological actions of scorpion ***L. quinquestriatus*** venom (Omran ***et al.***, 1992a,b and 1994; Omran and Abdel-Rahman, 1992 and 1994; Gwee ***et al.***, 1996; Fatani ***et al.***, 1998; Tarasiuk ***et al.***, 1998; Omran and McVean, 2000) there is no reports about its effect on tumors.

Cobra venom consist of three major proteins, cardiotoxins, neurotoxins and phospholipase A2 (Tu, 1977). Although these basic proteins are chemically similar, they exhibit different types of pharmacological actions including, cardiotoxic, cytotoxic and potent neurotoxic activities (Jun-Ling and William, 1991). The main lethal component of cobra venom is cardiotoxin, which is a strongly basic polypeptide containing many basic and hydrophobic amino acid residues. Its toxic effects generally involve membrane depolarization and ultimate cytolysis in many types of tissues, it has also been referred to as direct lytic factor (Shou-Jian and Chiu-Yin, 1996; Wen-Guey, 1997). The cytotoxic action on tumor cell lines of peptides from cobra venoms is reported by some investigators (Harvey, 1985; Dufton and Hider, 1988; Robert ***et al.***, 1993).

Scorpion ***L. quinquestriatus*** and the Egyptian cobra ***Naja haje*** are widely distributed in Egypt and the Middle East. Recently, we reported that venom from both animals induced ***in vitro*** apoptosis and necrosis to a permanent cell line of primary human embryonic kidney cells (293T) and a mouse myoblast (C2C12) cell line (Omran, 2002; Omran ***et al.***, 2002). The overall purpose of this study was to determine the potential efficacy, the cytotoxic and antitumor effects of ***L. quinquestriatus*** and ***Naja haje*** venoms, two potent toxins, on two established human breast and four prostate cancer cell lines.

Materials and Methods

Venom collection

Scorpions of species ***L. quinquestriatus*** were captured from Southern Egypt. Captive scorpions were kept alive in separate containers and venom collected according to the method described by Omran and McVean (2000). ***N. haje*** crude venom was milked from snakes collected from El-Fayoum region in Egypt. The venom was rapidly frozen, lyophilised and kept in a desiccator at 4°C until time of use.

Cells and media

The six cancer cell lines used in this study were a gift from Dr. Badar Usmani, Leeds University, UK. All cell lines are metastatic where, two cell lines represent human breast carcinoma (T47D and MDAMB-468) while the other four are human prostate carcinoma (DU145, PC-3, TSUpr1, LNCaP). They were maintained as monolyers cultured at 37°C in closed Falcon plastic dishes containing media supplemented with 10% heat inactivated fetal bovine serum (FBS). Cells were routinely grown in the growth medium RPMI 1640 (HyClone, Cramlington, UK) which was supplemented with 1 mM sodium pyruvate and 1 mM L-glutamine and also contained antibiotics (10,000 units/L penicillin and 100 mg/L streptomycin).

Venom cytotoxicity

The cells used in this study were harvested by trypsination (trypsin/EDTA for 2 min), resuspended in fresh serum free medium (SFM) and plated (3000-5000 cells/well) in 96-well flat micrometer plates. After 48 h, various concentrations (20, 50 and 100 μg/ml SFM) of both scorpion and cobra venoms were added and the cells were incubated for 24 h at 37°C with 5% CO_2. Media were collected at different time points (2, 4, 8 and 24 h) following incubation of both venoms with cancer cell lines and cell mortality (% of cell death) was determined using the CytoTox 96® (Promega, Madison, WI, USA) Non-Radioactive cytotoxicity assay. This assay accurately and rapidly measure cell death by quantitating the release of lactate dehydrogenase (LDH) a stable cytosolic enzyme from lysed cells (Cik ***et al.***, 1994; Hawryluk and Hirshfield, 2002). LDH was used as a cytotoxicity marker of some cancer cell lines treated with different anticancer agents (Li ***et al.***, 1995; Kumi-Diaka, 2002). The effect of scorpion and snake venoms on cancer cells survival was determined at each concentration from at least three different experiments with 6 replicate samples. This data was used to calculate the 50% lethal concentration (LC_{50}) for both venoms according to the method described by Maristela ***et al.*** (1999). Morphological and pathological alterations to all treated cell lines were monitored during the incubation period to follow up the histopathological changes and cell damage occurred to the different carcinoma. This was investigated qualitatively using a light phase contrast microscope (Leach DMIRB; Germany) and photos taken at different time intervals by computerised colour coolview camera using the Image-Pro® Lite software (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

Data were analyzed by ANOVA and the statistical probability of $P<0.05$ was considered significant.

Results

The potency of both, scorpion ***L. quinquestriatus*** and Egyptian cobra venoms as an inhibitor of cell proliferation and cytotoxic agent to the tested cancer cell lines is reflected by the LC_{50} values shown in Table 1. It is clear from these data that snake ***Naja haje*** venom is more potent as antitumor agent than scorpion venom against both breast and prostate cancer cell lines. LC_{50} values of cobra venom showed great variation in the two breast cancer cell lines, being 18 μg/ml in T47D cells and 63 μg/ml in MDAMB468 cells (almost three folds). Also, there is a great difference between the cytotoxic effect of cobra venom (LC_{50}= 18 μg/ml) and that of scorpion venom (LC_{50}= 62 μg/ml) on the T47D breast cancer cell line. On the other hand, the range of the LC_{50} values in case of prostate cancer cell lines were narrow (between 38 and 61 μg/ml) except for LNCaP cells where it was more than 100 μg/ml.

Table 1: The 50% lethal concentration (LC_{50}) of scorpion *Leiurus quinquestriatus* and Egyptian cobra *Naja haje* venoms (µg/ml) inducing cell death in six human cancer cell lines

	LC_{50} (µg/ml)	
Cancer cell line	Scorpion venom	Cobra venom
Breast T47D	62	18
MDAMB-468	>100	63
Prostate DU145	>100	38
PC-3	>100	42
TSUpr1	>100	61
LNCaP	>100	>100

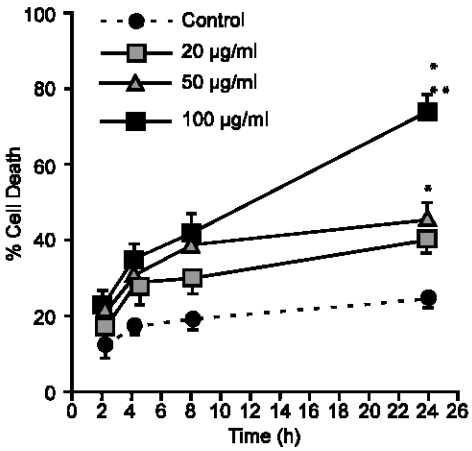

Fig. 1: The percentage of cell death induced by scorpion *L. **quinquestriatus*** venom on human breast cancer T47D cell line. Cultured cell line was incubated with varying doses (20, 50 and 100 µg/ml) of scorpion venom in serum free medium (SFM) for 24 h at 37°C. Control culture was treated with an equal amount of SFM. Values are means±S.E of 6 replica/treatment. Data were analyzed by one way ANOVA and statistical probability of $P<0.05$ was considered significant

* Significantly different compared to control cultures (untreated)

** Significantly different compared to 20 µg/ml dose

Fig. 1 shows the effect of different concentrations of scorpion venom on the percentage of cell death in breast cancer cell line (T47D). Incubation of T47D cells with *L. **quinquestriatus*** venom for 24 h induced non-sigificant effect during the first 8 h of treatment with all doses. However a significant increase in the number of dead cells was recorded after 24 h at 50 and 100 µg/ml doses compared to the control values. There was also a significant difference between the number of dead cells 73% induced by the highest dose and that takes place 44% at 50 µg/ml dose. Morphologically, dying cells showed fragmentation, condensation of their contents concomitant with shrinkage and appearance of vacuoles between adherent cells (Plate 1).

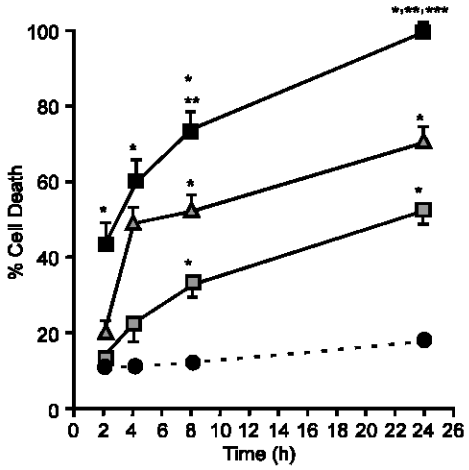

Fig. 2: The percentage of cell death induced by Egyptian cobra (***Naja haje***) venom on human breast cancer T47D cell line. More details are shown in Fig. 1.
* Significantly different compared to control cultures (untreated)
** Significantly different compared to 20 µg/ml dose
*** Significantly different compared to 50 µg/ml dose

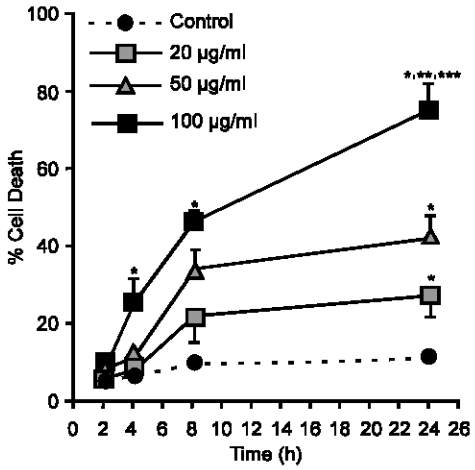

Fig. 3: The percentage of cell death induced by Egyptian cobra (***Naja haje***) venom on human breast cancer MDAMB-468 cell line. More details are shown in Fig. 1 and 2

Egyptian cobra venom inhibited cell proliferation and produced significant cell destruction of the breast cancer cell line (T47D) in a dose-related manner (Fig. 2 and Plate 2). A significant cell death occurred as early as 2 h after incubation with the highest venom dose (100 µg/ml) and after 4 h from treatment with 50 µg/ml dose. After 24 h from treatment with 20, 50 and 100 µg/ml

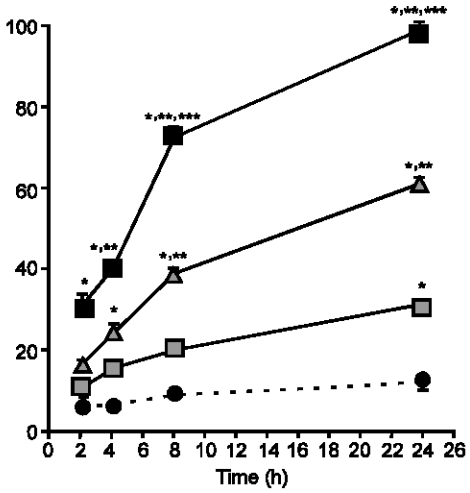

Fig. 4: The percentage of cell death induced by Egyptian cobra (***Naja haje***) venom on human prostate cancer DU145 cell line. More details are shown in Fig. 1 and 2

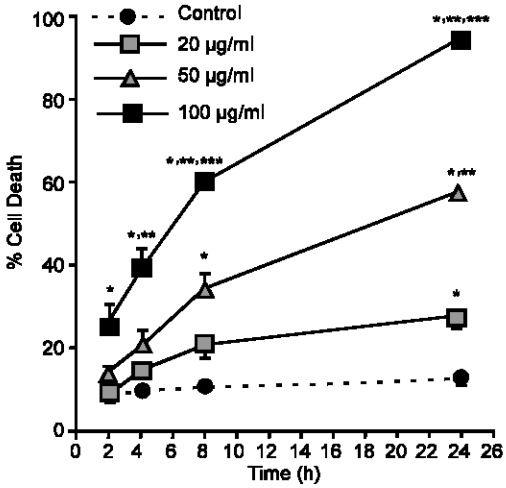

Fig. 5: The percentage of cell death induced by Egyptian cobra (***Naja haje***) venom on human prostate cancer PC-3 cell line. More details are shown in Fig. 1 and 2

snake venom, 52, 70 and 98% of the total number of cells were died, respectively. Plate 2 shows that cell vaculation and cellular content condensation was very clear at the lowest venom dose. Treatment with 50 µg/ml dose lead to the aggregation of dense irregularly shaped cellular debris (Plate 2C) and no intact cells were recognized in 100 µg/ml medium, which indicate the occurrence of severe cell death (Plate 2D).

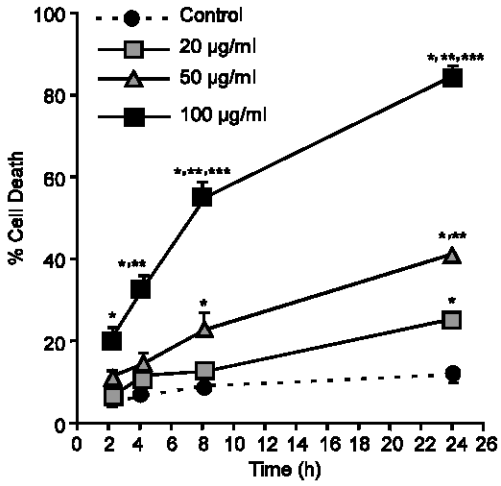

Fig. 6: The percentage of cell death induced by Egyptian cobra (***Naja haje***) venom on human prostate cancer TSUpr1 cell line. More details are shown in Fig. 1 and 2

A dose dependent cytotoxic effect was observed after incubation of breast cancer cell line MDAMB-468 with different concentrations of ***Naja haje*** venom (Fig. 3). 27 and 42% of treated carcinoma were injured after 24 h form addition of 20 and 50 µg/ml doses, respectively. These values were significantly different compared to the control data. A significant cell death occurred after 4 h 27% and persisted up to 24 h 75% at the highest dose that was significant compared to the other tested doses. Various structural abnormalities were recorded as a result of incubation with different concentrations of the venom. Cells were swollen before they destruct and cellular debris was observed in the media with floating dead cells (Plate 3D).

As shown in Fig. 4, incubation of prostate cancer cell line DU145 with variable doses of cobra venom produced a pronounced dose and time-dependent cytotoxic effect. The lowest venom dose induced significant cell death 31% after 24 h of treatment. In contrast, the medium tested dose (50 µg/ml) caused severe damage which started to be significant after 4 h and increased with time until reached 61% after 24 h. The highest dose killed almost all the prostate cancer cell 98% after 24 h of exposure, as also occurred with the breast cancer cell line T47D. This value and the data recorded after 8 h were statistically different compared to the other doses. Morphologically, the lowest dose (20 µg/ml) of the venom showed that most of the cells lost their characteristic appearance and coalesced together to the extent that the individual cell can not be identified. The coalesced cells displayed a syncitum-like appearance concomitant with an increase in the density of their cellular content. Only a few cells preserved the normal structure (Plate 4B). Also, the occurrence of some cellular swelling and certain areas devoid of any cultured cells (vacuoles-like) were observed in-between the coalesced cells at 50 µg/ml dose (Plate 4C).

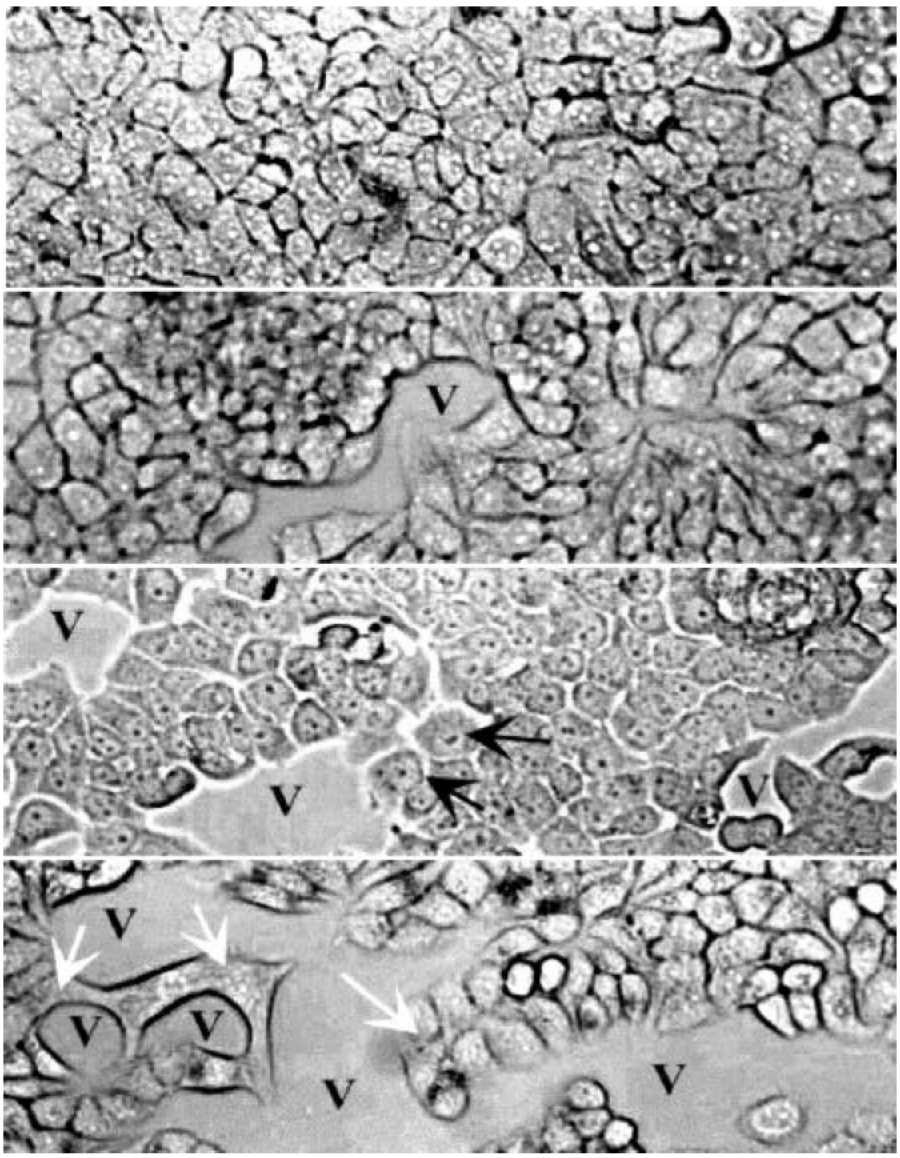

Plate 1: Light micrographs (phase contrast) of cultured human breast cancer T47D cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of scorpion *L. quinquestriatus* venom in serum free medium (SFM) at 37° C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field. The cells exhibited polygonal shape with distinct boundaries and homogenous or slightly granulated cellular contents

B: Cells incubated with 20 µg/ml dose. Note the presence of a few vacuoles like areas (white arrows, V) devoid of any cultured cells

C: Note the occurrence of some cellular swelling (black arrows) after incubation with 50 µg/ml dose and the density of the cellular contents was increased. The number of vacuoles was also increased

D: Cells incubated with the highest dose (100 µg/ml) of scorpion venom showed similar changes with more severity. Some cells were ruptured (D) and lost the continuity of their surrounding membranes

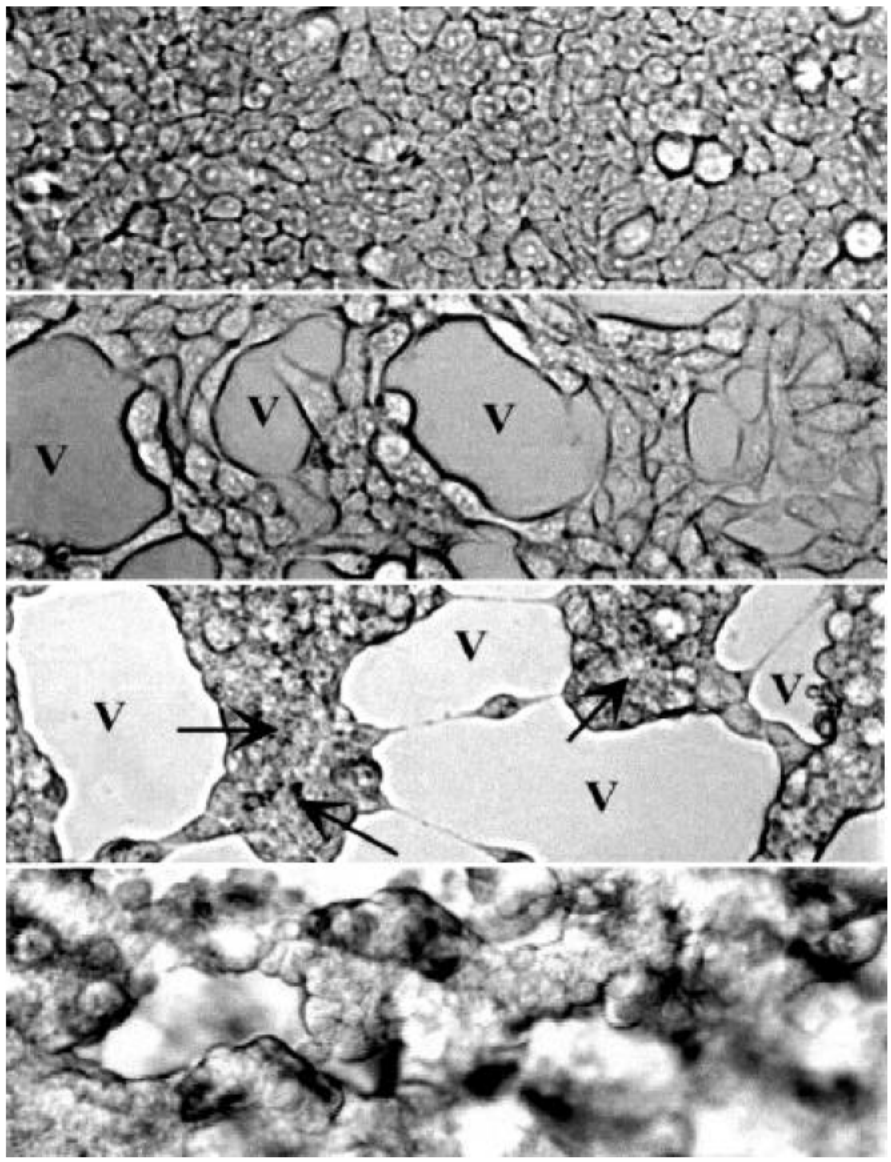

Plate 2: Light micrographs (phase contrast) of cultured human breast cancer T47D cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of Egyptian cobra (*Naja haje*) venom in serum free medium (SFM) at 37 °C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field

B: Cells incubated with 20 µg/ml dose. Note the presence of a some wide vacuoles (V) devoid of any cultured cells

C: The density of the cellular contents was increased after incubation with 50 µg/ml dose and Some cells were ruptured (black arrow) and lost the continuity of their surrounding membranes and the size of vacuoles (V) were also increased

D: Cells incubated with the highest dose (100 µg/ml) of cobra venom. Note that the cells are highly damaged and the remnants of cell debris were seen floating in the culture medium

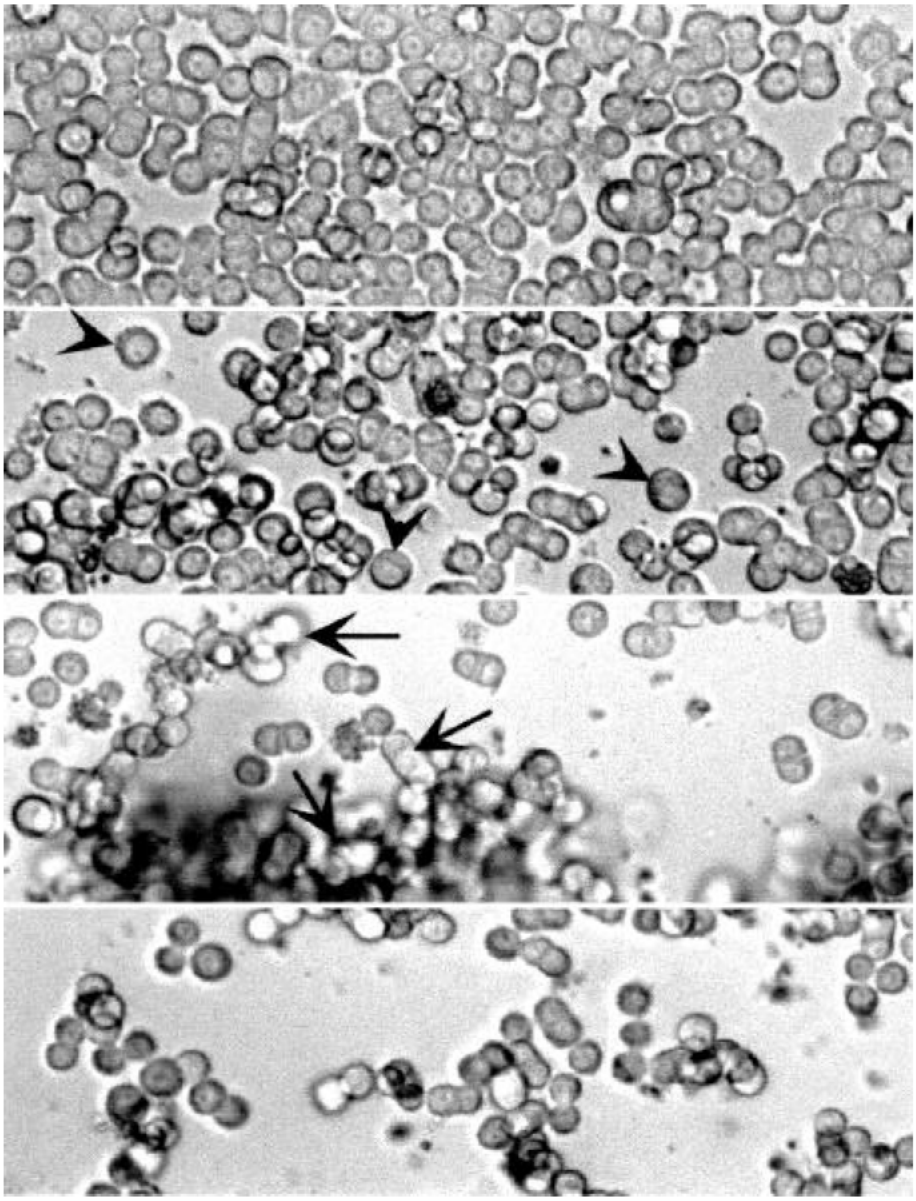

Plate 3: Light micrographs (phase contrast) of cultured human breast cancer MDAMB-468 cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of Egyptian cobra (*Naja haje*) venom in serum free medium (SFM) at 37° C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field. Cells are more or less rounded to oval in shape

B: Cells incubated with 20 µg/ml dose. Note the occurrence of some cellular swelling (arrows)

C: The density of the cellular contents was increased after incubation with 50 µg/ml dose and Some cells were ruptured (black arrow) and the remnants of cell debris were seen floating in the culture medium

D: Cells incubated with the highest dose (100 µg/ml) of cobra venom. Note that the cells are highly damaged

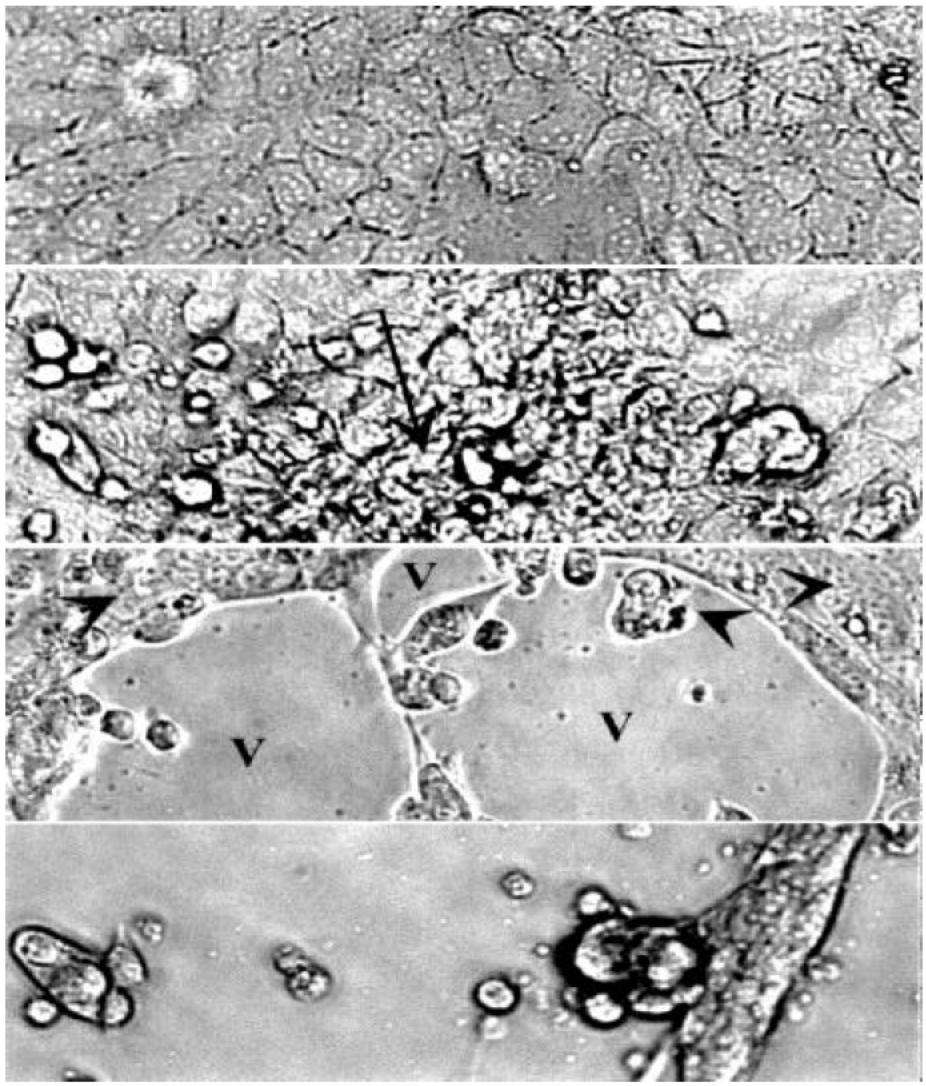

Plate 4: Light micrographs (phase contrast) of cultured human prostate cancer DU145 cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of Egyptian cobra (*Naja haje*) venom in serum free medium (SFM) at 37° C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field

B: Cells incubated with 20 µg/ml dose. Note that most of the cells lost their characteristic appearance and coalesced together to the extent that the individual cell can not be identified. The coalesced cells displayed a syncitum-like appearance concomitant with an increase in the density of their cellular content. Only a few cells preserved the normal structure

C: The occurrence of some cellular swelling and certain areas devoid of any cultured cells (vacuoles-like) were observed in-between the coalesced cells after incubation with 50 µg/ml dose. Some cells were ruptured (black arrow) and the remnants of cell debris were seen floating in the culture medium

D: Cells incubated with the highest dose (100 µg/ml) of cobra venom. Note that most of the cells are severely damaged and disappeared from the field

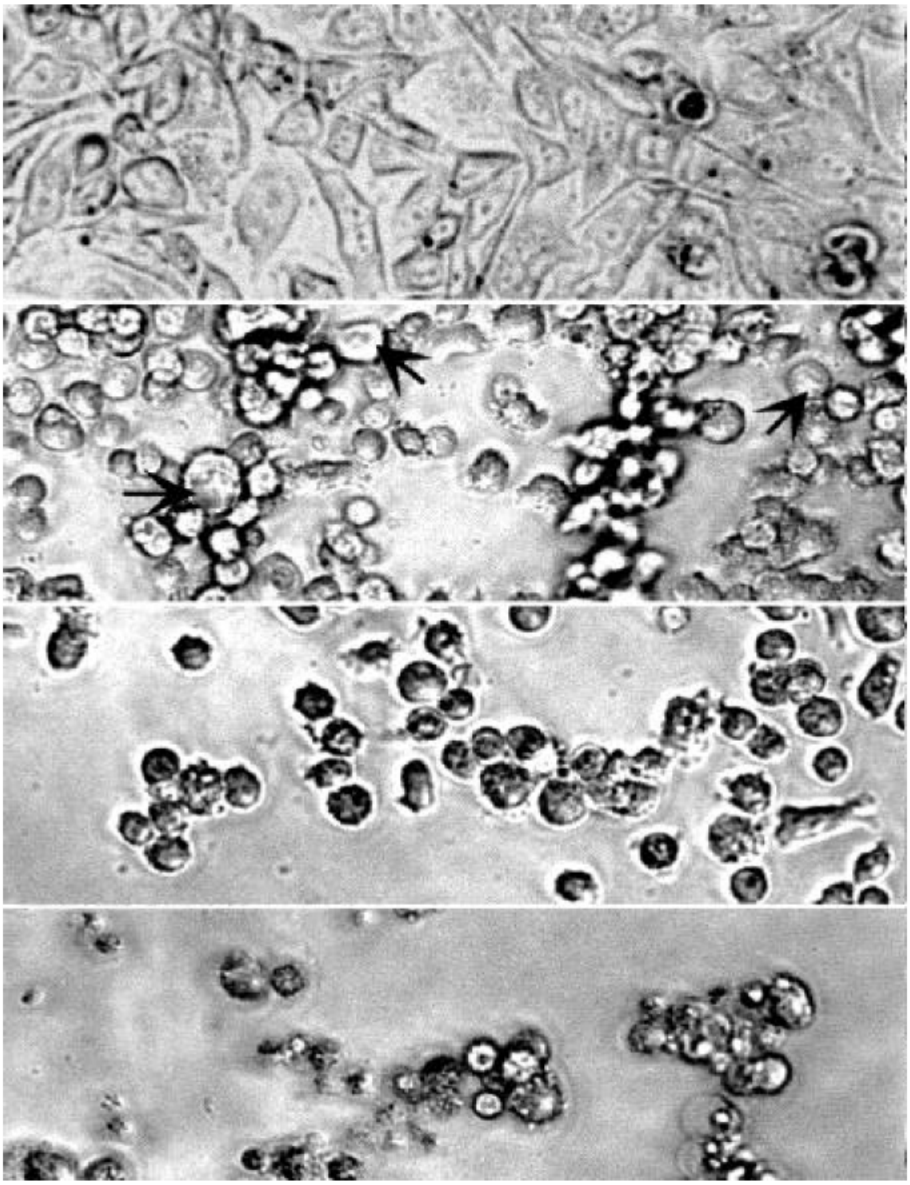

Plate 5: Light micrographs (phase contrast) of cultured human prostate cancer PC-3 cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of Egyptian cobra (*Naja haje*) venom in serum free medium (SFM) at 37° C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field

B: Cells incubated with 20 µg/ml dose. Note that most of the cells lost their characteristic appearance with an increase in the density of their cellular content. Note the occurrence of some cellular swelling (arrows)

C: Severe cell damage and the remnants of cell debris were observed after incubation with 50 µg/ml dose

D: Cells incubated with the highest dose (100 µg/ml) of cobra venom. Note that dead cells are floating in the culture medium

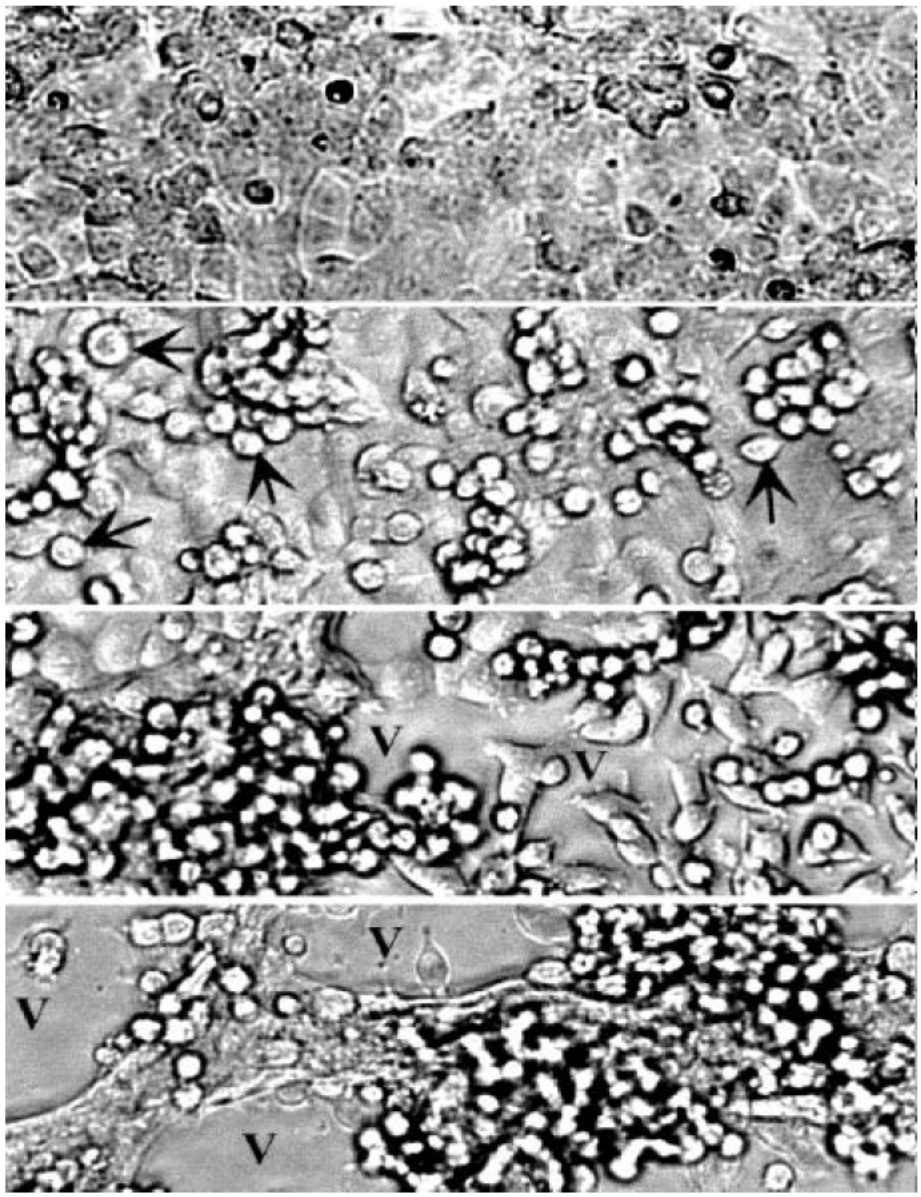

Plate 6: Light micrographs (phase contrast) of cultured human prostate cancer TSUpr1 cell line after 24 h of incubation with varying doses (20, 50 and 100 µg/ml) of Egyptian cobra (*Naja haje*) venom in serum free medium (SFM) at 37°C. (X 400)

A: Untreated control cells were homogeneously distributed in the culture field

B: Cells incubated with 20 µg/ml dose. Note that most of the cells lost their characteristic appearance. Note the occurrence of some cellular swelling (arrows)

C: Some cells were ruptured and lost the continuity of their surrounding membranes and the numbers of empty-looking areas were observed after incubation with 50 µg/ml dose

D: Cells incubated with the highest dose (100 µg/ml) of cobra venom. Severe cell death with more empty looking areas

The anticancer effect of cobra venom on prostate cancer cell line PC-3 is illustrated in Fig. 5 and Plate 5. At a dose of 20 μg/ml, ***Naja haje*** venom killed 28% of the cells after 24 h, whereas 50 μg/ml dose destroyed 35 and 59% of the prostate tumor after 8 and 24 h, respectively almost similar to the cytotoxicity observed on cultured prostate carcinoma DU145. These values were significantly different compared to the control data. The number of cells damaged by the snake venom at 100 μg/ml dose increased with incubation time, but the effect started to be significant after only 2 h of incubation. About 61% of cells recognized dead after 8 h and the maximal cell destruction 94% was recorded after 24 h, which were statistically significant compared to the control and the other tested concentrations. The same morphological alterations induced by Egyptian cobra venom on DU145 prostate cancer cells were also observed in case of PC-3 prostate carcinoma (Plate 5).

Naja haje venom produced significant anticancer effect in the culture at the different tested concentrations compared to the control TSUpr1 prostate cancer cell line (Fig. 6). The maximal cytotoxic effect recorded after 24 h at 20 and 50 μg/ml doses was 25 and 41%, respectively while it reached to 84% after incubation with 100 μg/ml dose. At the same time, the highest venom dose induced significant cancer cell death at all time points of incubation. The morphological changes induced by snake venom on TSUpr1 cells are illustrated in Plate 6. The occurrence of some cellular swelling and presence of a few empty looking areas (vacuoles like) devoid of any cultured cells was observed after treatment with 20 μg/ml dose. Cellular swelling was increased after incubation with 50 μg/ml snake venom (Plate 6C) and the density of the cellular contents was also increased. Some cells were ruptured and lost the continuity of their surrounding membranes and the numbers of empty looking areas were also increased. Cells incubated with the highest dose (100 μg/ml) of cobra venom (Plate 6D) had observed similar changes with more severity. It is very important to mention that the cytotoxic effect of Egyptian cobra venom on all prostate cancer cell lines in this study was dose and time-related.

Discussion

The WHO estimates that over the next 25 years there will be over 100% increase in the number of people who develop cancer in 31 countries. By the year 2020 the number of new patients each year will be a frightening 20 million. Cancer is thus becoming a major health problem all over the world (Sikora, 1999b; Sikora ***et al.***, 1999). Treatment of cancer involved different clinical protocols, some of them used in combination, including surgery, chemotherapy, radiotherapy, gene therapy and some recent immunological approaches. All of these protocols has its own advantages and disadvantages and mainly depend on the type of tumor and the stage of the disease especially in patients with a variety of metastatic tumors.

This study shows for the first time that crude venoms from scorpion ***L. quinquestriatus*** and Egyptian cobra could be potent anticancer agents. The damage induced to the breast and

prostatic cancer cell lines by both venoms is direct and is not secondary to disruption of the microcirculation, which causes histopathological and cytological changes in animals and humans (Arce ***et al.***, 1991). The cytotoxicity and the anticancer effect were remarkable and cell survival was highly reduced at the highest tested concentration (100 µg/ml). Because of the fast effects that observed and the lysis of the plasmalemma and/or organelle membranes that immediately causing the greatest level of cell population death as evident by significant increase in the cytosolic LDH release, we suggest that these toxins act at the membrane level of the tumor cells. Numerous previous investigations (Mebs and Ownby, 1990; Balboni ***et al.***, 1992; Bruses ***et al.***, 1993; Omran, 2002; Omran ***et al.***, 2002) have reported the same loci of action for these venoms and some other natural toxins. Further more, Bruses ***et al.*** (1993) mentioned that some myotoxins that isolated from snake venom either move through pores or channels in the membranes or cross the sarcolemma by endocytosis, which allowing the passage of ions down their concentration gradient, resulting in osmotic changes in organelles, followed by several unidentified mechanisms leading to cell death.

It was reported that some crude scorpion venoms have a very profound histopathological and necrotic effects in humans and animals (Yarom and Braun, 1969; Tu, 1977; Omran ***et al.***, 1994; Tarasiuk ***et al.***, 1998). However, there are no previous extensive experiments on the effect of these potent venoms on tumors. In this investigation, although scorpion ***L. quinquestriatus*** venom kills breast cancer T47D cells significantly 73% within 24 h especially after incubation with the highest venom dose with LC50 value equal to 62 µg/ml, it has non-significant anticancer effect on the other tested cancer cell lines. This might reflect a specific selectivity of scorpion venom for this carcinoma than for the other types of cancer cells. This effect could be mediated through particular unknown receptor(s) located on the plasma membrane or in the cytosole of the breast cancer T47D cells that initiate a series of cellular events leading to cell death. This postulation is in part in agreement with the reports of Bruses ***et al.*** (1993) which mentioned the possibility that some toxins must bind to a specific receptor in the membrane, before they can exert their action. This could explain the high selectivity of scorpion toxins for the breast cancer T47D cell line. This postulation needs more experimental confirmation.

Our data clearly demonstrated a remarkable ***in vitro*** cytotoxic effects and anticancer property of ***Naja haje*** venom against breast and prostate cancer cell lines. Mostly, these consequences were time and dose dependent in all cell lines. Cobra venom induced significant cell death of breast cancer cell line T47D (98%) and MDAMB-468 (75%) after 24 h incubation with 100 µg/ml. Also the highest snake venom dose killed 98, 94 and 84% of prostate carcinoma DU145, PC-3 and TSUpr1, respectively. It is important to emphasize that an early significant cell destruction, within 2-4 h was recorded in most cell lines treated with cobra venom even with the lower doses (20 and 50 µg/ml). Various pathological and structural abnormalities were recorded as a result of incubation with different concentrations of the venom. Dying cells

showed fragmentation, condensation of their contents concomitant with shrinkage and appearance of vacuoles between adherent cells, which were swollen before they destruct. Recently we (Omran ***et al.***, 2002) indicated that cobra venom induce ***in vitro*** cell death by two different mechanisms, necrosis and induction of apoptosis. This unusual form of cell death induced by ***Naja haje*** venom may represent a combination of apoptosis necrosis within the same cell. This kind of cell destruction was recognized by Shimizu ***et al.*** (2000). It can be suggested that cell injury caused by the cytotoxic components of the cobra venom activate a death program that in turn leads to irreversible damage and necrotic effects to the tested cancer cells.

It is well documented that venoms of snakes belonging to the genera ***Naja*** (including ***Naja haje***) contain active complex components such as cardiotoxins, cytotoxins, crotoxin and phospholipase A2 (Tu, 1977; Tonsing ***et al.***, 1983). Lorea ***et al.*** (1997) and Maristela ***et al.*** (1999) reported that lectin which is isolated from the snake venom is an effective ***in vitro*** inhibitor of cell growth of three human melanoma cell lines and eight human cancer carcinoma (Kidney, pancreas, prostate and colon) especially against renal and pancreatic cancer cell lines. Lectin like proteins have been found in the venom of four snake species, Elapidae, Viperidae, Crotalidae and Bothrops (Carvalho ***et al.***, 1998) and considered as a member of the cytotoxins. Furthermore, an extensive studies have been done in the last decade about the effectiveness, potency pharmacokinetics and safety of a combined natural product (crotoxin and cardiotoxin) derived from purified ***Naja naja atra*** snake venom as an anti-neoplastic agent (Newman ***et al.***, 1993; DeTolla ***et al.***, 1995; Costa ***et al.***, 1997; Costa ***et al.***, 1998; Stanchi ***et al.***, 2000; Costa ***et al.***, 2001). They reported that this natural compound which is known as VRCTC-310, represents a new membrane interactive anticancer agent which produces a unique spectrum of cytotoxicity ***in vitro*** and has demonstrated interesting ***in vivo*** antitumor efficacy. An 83% inhibition of tumor growth of mice bearing Lewis lung carcinoma was achieved on day 20 using an escalating daily intramuscular doses of VRCTC-310 starting at 1.8 mg/kg and reaching 6.3 mg/kg/day (Newman ***et al.***, 1993). Although some irritation around the sites of i.m. injection was noted, animal weight loss was negligible and there were no other signs of adverse toxicity. Also DeTolla ***et al.*** (1995) indicated that beagle dogs injected with this compound tolerated the subchronic treatment better than the acute and no clinical signs of neurotoxicity were observed. Interesting results were obtained by Costa ***et al.*** (1998) about the first communication of ***in vivo*** antitumoral activity of VRCTC-310 that injected locally to humans. The authors reported their clinical experience with this combined snake toxins agent in two patients suffering from advanced cancer in which the skin was severely compromised. The local (peritumoral) treatment with the drug (0.014 mg/kg/week during 6 weeks) provoked the complete disappearance of a relapsed skin squamous cell cancer in one patient. The other patient was an aged woman with local-advanced breast cancer who was inoculated intra-and-peritumoral with VRCTC-310. After 6 weekly courses (0.014

mg/kg/week) with the drug, more than 80% tumor reduction was seen. Follow up for 133 days demonstrated not only an objective complete response of the primary tumor mass but the disappearance of supraclavicular tumor masses as well as a significant reduction in lymphangitis. These promising results and the data obtained from this research further support the role of the natural products and toxins as antitumors agents. Development of new physiologically efficient and potent anticancer treatment would be useful in the clinical management of cancer. More research work should be done concerning characterization and identification of the antitumor venoms and their different efficient toxic components from the wide variety of venomous animals distributed all over the world without ignoring the fact that some of them are fatal if delivered in lethal doses. ***In vivo*** trials involves with the estimation of the effective anticancer doses (single or escalating) of these venoms, their safety profile, pharmacodynamics, pharmacokinetics and the rout of their administration (i.m. local, etc.) should be carried out.

In conclusion, results provides a rational for ***in vivo*** studies to determine whether scorpion *L. **quinquestriatus*** and Egyptian cobra venoms will provide effective therapy for breast and prostate cancer especially in the cases of metastasis instead of the available chemotherapy and radiotherapy, which induce unwanted side effects.

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