Original Article

Evaluation of the Cytotoxic Effects of *Odontobuthus doriae* Crude Venom on the MCF-7 Breast Cancer Cell Line

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Abstract

Background: Scorpion venom is a complex mixture containing toxic peptides, free amino acids, enzymes, nucleotides, lipids, amines, mucoproteins and other bioactive components. It has been reported to exhibit a range of medicinal properties, including anticancer, antithrombotic, anticoagulant, fibrinolytic, analgesic, antitumor and antiepileptic effects. This study aimed to evaluate the anticancer effects of crude venom from *Odontobuthus doriae* on the Michigan Cancer Foundation-7 (MCf-7) breast cancer cell line.

Methods: 2×10^4 MCF-7 cancer cells were cultured in T25 flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. After overnight incubation, the culture medium was replaced with different concentrations of crude venom (0.2, 0.48, 0.97, 1.95, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500 µg/mL). The cytotoxic effects were assessed using the MTT reduction assay at 24, 48 and 72 hours post-treatment, performed in triplicate. Absorbance was measured at 570 nm using an ELISA reader.

Results: A concentration-dependent decrease in cell viability was observed. A statistically significant difference in cytotoxicity was observed between the 24 hour and the 48/72-hour treatments, while no significant difference was noted between the 48 and 72 hour time points. The IC_{50} values were calculated to be 4.775 µg/mL (24 h), 31.87 µg/mL (48 h), and 3.543 µg/mL (72 h).

Conclusion: The crude venom of *O. doriae* exhibits significant cytotoxic effects against MCF-7 breast cancer cells in a dose- and time-dependent manner, suggesting its potential as a natural anticancer agent.

Keywords: *Odontobuthus doriae*; Scorpion venom; Breast cancer; MCF-7 cell line; Cytotoxicity

Introduction

In recent years, cancer has emerged as a major global health concern, affecting millions of people worldwide (1). According to the World Health Organization, cancer is the second leading cause of death globally, accounting for approximately 9.6 million deaths annually (2). Despite significant advances in diagnosis and treatment, cancer-related mortality remains high.

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Among various cancers, breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related death among women (4–17). During the past recent years, various research studies have shown that novel therapeutic approaches are urgently needed (18). Traditional treatments such as chemotherapy, radiotherapy and surgery can be effective, but often result in systemic toxicity and drug interactions due to a lack of selectivity (19). Since 1987, over 1,000 chemical compounds have undergone preclinical evaluation in chemopreventive testing programs. Although these agents show promise in terms of efficacy and mechanism of action, many exhibit adverse effects on vital organs such as the nervous system, heart, liver, bladder, lungs and kidneys (2). Therefore, there is growing interest in identifying alternative, naturally derived agents, particularly those from animal venoms, for the prevention and treatment of cancer (12).

Invertebrate venom, despite its inherent toxicity, is a promising source of bioactive compounds with significant anticancer properties. Historically, traditional medicine in Asia, Africa and other regions has used these venoms to treat a variety of diseases, especially those obtained from scorpions, wasps, hornets and cone snails (20).

The use of scorpion venom in traditional medicine dates back thousands of years, especially in regions such as China, India and Africa (6–12). Scorpion venom is a rich and complex mixture containing toxic peptides, free amino acids, enzymes, nucleotides, lipids, amines, mucoproteins and heterocyclic compounds. These components are known to exert diverse pharmacological activities, including anticancer, antithrombotic, anticoagulant, fibrinolytic, analgesic, antitumor and antiepileptic effects (9–23).

A growing body of in vitro and in vivo studies has demonstrated that scorpion venoms can inhibit cancer progression and induce apoptosis (2). These venoms exhibit unique mechanisms of action, such as blocking ion channels, enhancing caspase activity and arresting the cell cycle at G1, S, or G2 phases (1–7). Additionally, they induce oxidative stress within cancer cells, leading to the production of reactive oxygen species (ROS) and alterations in redox balance, which are critical factors contributing to DNA damage and cell death (14–16). These oxidative shifts may also influence the sensitivity of cancer cells to chemotherapeutic agents (11).

Scorpions have existed for over 400 million years and are distributed across diverse ecosystems. To date, 2540 scorpion species in 21 families have been described worldwide. In Iran, at least 68 valid species from four families have been reported, among which the family Buthidae shows the highest diversity and abundance. Among them, members of the family Buthidae, especially those from the genus *Odontobuthus*, are considered highly dangerous. The genus includes four species: *O. bidentatus*, *O. doriae*, *O. tavighiae* and *O. tirgari*. For the first time, *O. tavighiae* was recorded from Fars Province and *O. tirgari* from South Khorasan Province (24). The yellow scorpion *O. doriae*, commonly found in central and southern Iran, is known for its potent venom. Its sting can cause a range of symptoms from localized pain and inflammation to necrosis and, in severe cases, muscle paralysis, particularly in children (3). Notably, previous research has shown that *O. doriae* venom exhibits strong cytotoxic effects on ion channel-expressing cell lines, including neuronal cells (3).

In this study, we evaluated the cytotoxic effect of crude *O. doriae* venom on the MCF-7 breast cancer cell line. While many studies have explored the anticancer properties of scorpion venom in vitro, the translation of these findings into effective tumoricidal therapies remains limited. Comprehensive in vivo studies are urgently needed to further elucidate the potential of scorpion venoms as anticancer agents. Ultimately, the development and commercialization of venom-derived anticancer drugs will depend on their ability to selectively target tu-

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mor cells without inducing toxicity in normal human tissues during clinical trials (7).

Materials and Methods

Venom preparation

Crude lyophilized venom of *Odontobuthus doriae* was obtained from Razi Vaccine and Serum Research Institute (RVSRI), Karaj, Iran, reconstituted in sterile double-distilled water at 8 mg/ml, and centrifuged at 10,000×g to separate mucus and other insoluble components. The supernatant was carefully collected, aliquoted into 0.5 mL vials, and stored frozen for further use. Following the determination of the initial concentration, a series of venom dilutions was prepared for cytotoxicity testing. Its protein content was measured by the BCA (bicinchoninic acid) protein method.

Determination of crude venom protein concentration

The protein concentration of the crude venom was measured using the BCA (bicinchoninic acid) protein assay kit (BioAida), following the manufacturer's instructions. First, the standards were made based on BSA. First, we added 25 microliters (µl) of each of our samples to the wells of a 96-well microplate. Also, we added 25 microliters (µl) of BSA (bovine serum albumin) standard to one well. After adding samples and standards, we added the working solution to each well. After adding the working solution, we incubated the wells for 60 minutes at 60 °C. Finally, we measured the optical absorbance (OD) at 562 nm. A standard curve was generated using bovine serum albumin (BSA) for each new plate (Fig. 1).

MCF-7 cell culture

The MCF-7 breast cancer cell line was obtained from the Department of Immunology, Tehran University of Medical Sciences. Cells were cultured in T25 tissue culture flasks containing Dulbecco's Modified Eagle Medium (DMEM, BioAida), supplemented with 10% fetal bovine serum (FBS, DNAbioTech) and 1% penicillin-streptomycin (10,000 IU/mL and 10,000 µg/mL; BioAida). Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 and 80% humidity. The culture medium was refreshed three times per week. Cell density was determined using a hemocytometer, and 2×10^4 cells were seeded into each well of a 96-well plate for subsequent experiments.

Cytotoxicity assay of crude venom on MCF-7 cells

To assess the cytotoxic effect of the crude venom, 2×10^4 MCF-7 cells were seeded into each well of a 96-well plate containing 100 µL of complete medium and incubated overnight under optimal conditions. The next day, the medium was removed and replaced with 100 µL of fresh medium containing various concentrations of *O. doriae* venom (0.2, 0.48, 0.97, 1.95, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500µg/mL). Plates were incubated for 24, 48 and 72 hours at 37 °C. Morphological changes in cells were observed and documented using an inverted microscope.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after 24, 48 and 72 hours of venom exposure. In each well, the medium was replaced with 100 µL of MTT stock solution (5 mg/mL in PBS), and plates were incubated for 4 hours at 37 °C. Subsequently, 100 µL of dimethyl sulfoxide (DMSO, DNAbioTech) was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader or an enzyme-linked immunosorbent assay (ELISA) reader. All experiments were conducted in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to evaluate significant differences between treatment groups.

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Data analysis

Values were presented as means ± standard deviation (n=3). The one-way ANOVA was used to compare the difference between the experiment and control groups. Statistical significance was indicated as follows: *p≤ 0.05, **p≤ 0.01 and ***p≤ 0.001 (A p-value of ≤ 0.05 was statistically significant). Also, Dunnett's was designed to compare different concentrations against controls, and Tukey was used for all pairwise comparisons. Inhibitory concentration (IC_{50}) was estimated using the Graphpad PRISM9 method and a dose-response curve, respectively.

Results

Determination of protein concentration of crude venom

Using the BCA protein assay kit, the absorbance value for the crude *O. doriae* venom was measured at 1.57. This corresponded to a protein concentration of 895 µg/mL, which was used as a stock concentration for preparing different dilutions in subsequent cytotoxicity experiments. Figure 1 shows the calibration curve. Absorbance values were measured at 562 nm using the Bicinchoninic Acid (BCA) assay, and a standard curve was generated using bovine serum albumin (BSA) as the reference protein. The BCA Kit Protein concentration determination showed that absorption of the initial concentration of crude *O. doriae* venom was 1.57. It means that the concentration of the sample was 895 $\mu g.mL^{-1}$.This data was used as a standard for the next steps.

Determination of the cytotoxicity of crude venom on MCF-7 cancer cells

Table 1 shows the viability of MCF-7 breast cancer cells following treatment with various concentrations of crude *O. doriae* venom compared to the untreated control group. At the 24 hour time point, a clear dose-dependent decrease in cell viability was observed. As the venom concentration increased, the survival rate of cancer cells consistently declined. The lowest viability recorded at this time was 30.73%, observed at concentrations of 250 and 500 µg/ mL. In contrast, the 48 and 72 hour time points did not follow a consistent dose-response trend. Although decreased viability was still observed at certain concentrations, a paradoxical increase in survival occurred at higher doses. The lowest viability at 48 and 72 h was 49.39% and 61.67%, respectively, both at the 125 µg/ mL concentration.

In Table 2, statistical analysis using one-way ANOVA revealed a significant effect of venom concentration on cell viability at 24 h (p= 0.0129), indicating a dose-dependent cytotoxic effect, whereas the differences at 48 h and 72 h were not statistically significant.

There is a significant difference between the 24 h group and the 48 and 72 hour groups (p< 0.05) (Fig. 2), while no significant difference was found between the 48 and 72 hour groups (Table 3). Therefore, a dose- and time-dependent decrease in cell viability was observed, comparing different concentrations at different times. A significant difference between the 24 h group and the 48 and 72 h groups, while no significant difference was found between the 48 and 72 hour groups. Data are presented as mean±SD of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (p< 0.05 indicates a significant difference compared to the control).

Determination of 50% inhibitory concentration (IC_{50})

The 50% inhibitory concentration (IC_{50}) values of *O. doriae* crude venom on MCF-7 cells were determined through regression analysis of the dose-response curves using GraphPad Prism version 8 (Fig. 3). The dilution range showed that the cytotoxicity of *O. doriae* venom on MCF-7 was higher than that of the control group (p< 0.05). Venom-treated cells clearly exhibited comet formation, whereas control cells did not. The IC_{50} values at 24, 48 and 72 h were calculated to be 4.775 µg/mL, 31.87 µg/mL

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and 3.543 µg/mL, respectively (Table 4). These results indicate a time-dependent variation in cytotoxic potency, with the most potent effect observed at 72 h, followed by 24 h, and the least potent effect at 48 h. Comparing each dose to control showed that doses of 62.5, 125, 250 and 500 µg/mL were significantly different from control. On the other hand, lower doses (0.24 to 31.25 µg/ mL) did not show significant differences from control (p> 0.05).

Table 1. Viability rate (%) of MCF-7 cancer cells after exposure to various concentrations of *Odontobuthus doriae* crude venom at different time points. Cell viability was assessed using the MTT assay and is expressed as a percentage relative to the untreated control group

	Viability rate (%)		
Concentration (µg.mL^{-1})	**24h**	**48h**	**72h**
0	100	100	100
0.24	75.41	100	95.59
0.48	84.86	93.03	76.21
0.97	84.16	90.6	78.19
1.95	80.61	79.69	85.9
3.9	83.92	76.36	90.08
7.81	53.42	67.27	83.92
15,62	52	73.03	79.51
31.25	43.02	77.87	74.77
62.5	35.93	79.69	65.85
125	34.98	49.39	61.67
250	30.73	74.24	65.19
500	30.73	81.18	70.48

Table 2. Statistical analysis of MCF-7 cell viability using one-way ANOVA at different time points following treatment with varying concentrations of *Odontobuthus doriae* crude venom

Source of Variation	SS	DF	MS	F statistic	p-value
Treatment	3089	2	1544	4.921	0.0129
Residual	11299	36	313.9		
Total	14388	38			

R-squared=0.215, SS=sum of square, DF=degree of freedom, MS=mean square

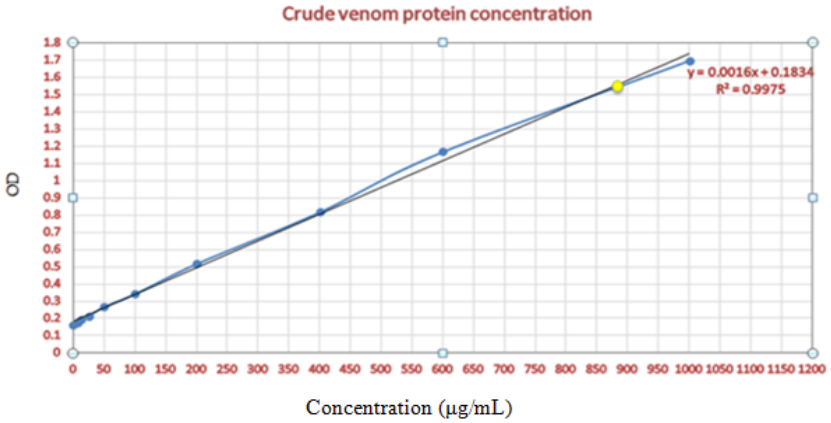

Fig. 1. Calibration curve for determining the protein concentration of *Odontobuthus doriae* crude venom dissolved in culture medium

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Table 3. Results of multiple comparisons test (Tukey's post hoc analysis) following one-way ANOVA to evaluate differences in MCF-7 cell viability between different concentrations of *Odontobuthus doriae* crude venom at 24, 48 and 72 hours. Significant differences ($p< 0.05$) were observed primarily at 24 h, confirming the dose-dependent cytotoxic effect of the venom during early exposure

Multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
24h vs. 48h	-19.43	-36.83 to -2.030	Yes	*	0.0245
24h vs. 72h	-18.28	-35.68 to -0.8765	Yes	*	0.0370
48h vs. 72h	1.153	-16.25 to 18.55	No	ns	0.9978

Table 4. Results of Dunnett's post hoc test to compare different concentrations against controls

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted p-value
control vs. 0.24	9.667	-27.66 to 47.00	No	Ns	0.9899
control vs. 0.48	15.30	-22.03 to 52.63	No	Ns	0.8403
control vs. 0.97	15.68	-21.65 to 53.01	No	Ns	0.8224
control vs. 1.95	17.93	-19.40 to 55.26	No	Ns	0.7033
control vs. 3.9	16.55	-20.78 to 53.88	No	Ns	0.7788
control vs. 7.81	31.80	-5.533 to 69.13	No	Ns	0.1259
control vs. 15.62	31.82	-5.510 to 69.15	No	Ns	0.1254
control vs. 31.25	34.78	-2.550 to 72.11	No	Ns	0.0775
control vs. 62.5	39.51	2.180 to 76.84	Yes	*	0.0339
control vs. 125	51.32	13.99 to 88.65	Yes	**	0.0035
control vs. 250	43.28	5.950 to 80.61	Yes	*	0.0169
control vs. 500	39.20	1.874 to 76.53	Yes	*	0.0358

Ns: non-significant, *: $p\leq 0.05$, **: $p\leq 0.01$

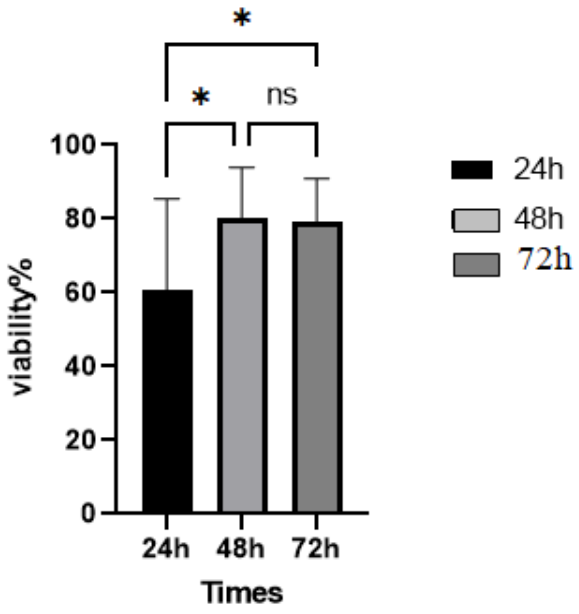

Fig. 2. Viability of MCF-7 cells after exposure to different concentrations of *Odontobuthus doriae* crude venom at 24, 48, and 72 hours. Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay

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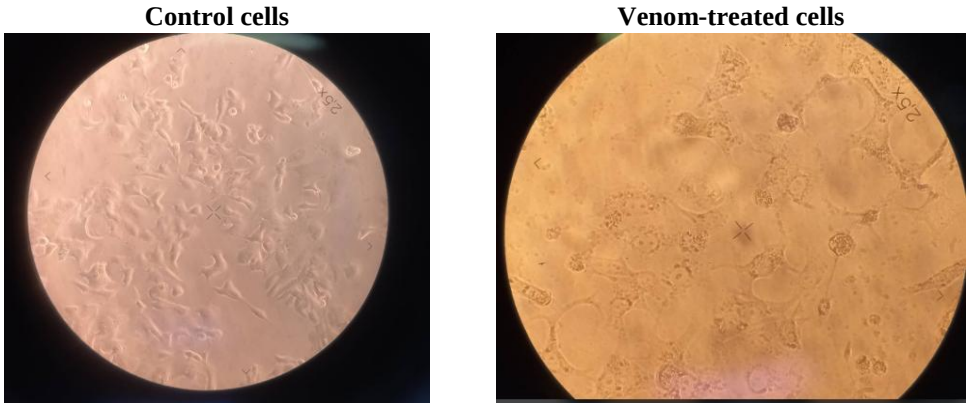

Fig. 3. The anticancer potential of crude *Odontobuthus doriea* venom was investigated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium)assay in this work using MCF-7 cell lines. Both healthy and apoptotic cells are shown in this figure

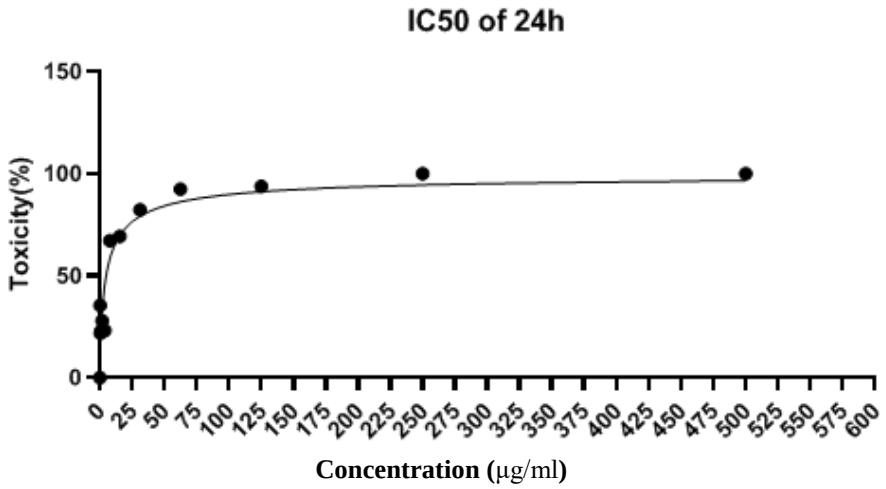

Fig. 4. The 50% inhibitory concentration (IC_{50}) values of *Odontobuthus doriae* crude venom on MCF-7 cells at 24 hours. Given that the survival of cancer cells at different concentrations of venom was observed and was significant in the first 24 hours, a toxicity graph was drawn and plotted for 24 hours

Discussion

Scorpion venom contains several compounds that several studies have shown that different scorpion species have effects on cancer cell lines and can act against cancer cells (1, 2, 22).

Some scorpion venoms can also reduce tumor growth by inducing antitumor and apoptosis, and even improve hematological parameters in some endocrine models (2). This is particularly observed in some cancer cells, although the extent of this effect depends on the scorpion and the cell species.

In Iran, the yellow scorpion, *O. doriae*, is considered a significant threat due to its potent neurotoxicity and severe pathophysiological effects. Researchers are trying to elucidate the underlying mechanisms of its neurotoxicity (3). The apoptotic and antiproliferative effects of *O. doriae* venom on human neuroblastoma cells were first reported by Zargan et al. (5). This study confirmed a concentration-dependent relationship between venom dose and cytotoxic effect. In our study, breast cancer cells

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(MCF-7) treated for 24, 48 and 72 hours with *O. doriae* venom concentrations ranging from 0.2 to 500 μg/mL exhibited decreased survival, with viability percentages of 100, 75.41, 84.86, 84.16, 80.61, 83.92, 53.42, 52.00, 43.02, 35.93, 34.98, 30.73 and 30.73%, respectively. Comparing each dose to control showed that doses of 62.5, 125, 250 and 500 μg/mL were significantly different from control. On the other hand, lower doses (0.24 to 31.25 μg/mL) did not show significant differences from control ($p> 0.05$). This aligns with previous studies showing a dose-dependent decrease in cell viability following scorpion venom exposure. Experimental evidence from several studies suggests that scorpion venom can lead to oxidative stress and activation of the apoptotic pathway. For example, Keshavarz et al. reported that the venom can disrupt cellular health, increase NO, and decrease catalase and GSH, leading to caspase-3 activation (11). This finding is consistent with other observations of reduced cancer cell viability and increased apoptosis in lines such as MCF-7 (1, 22).

Zargan et al. also found that *O. doriae* venom reduced DNA synthesis and induced apoptosis in MCF-7 cells, as demonstrated by increased LDH release, decreased levels of cellular antioxidants (GSH and catalase), and increased reactive nitrogen species (RNI) (15). Our study similarly demonstrated a concentration-dependent reduction in cell viability within the first 24 hours. Morphological changes consistent with apoptosis, such as swelling, membrane rupture and cellular aggregation, were also observed under a microscope. This supports our findings that *O. doriae* venom exhibits in vitro cytotoxicity in MCF-7 cells. These changes were associated with caspase-3 activation and nuclear DNA fragmentation, supporting the venom's potential as a source of antiproliferative and antiapoptotic compounds (15).

In a study comparing the effects of *O. doriae* and *Buthotus saulcyi* venoms on human cell lines, *O. doriae* venom induced dose-dependent cytotoxicity in the 1321N1 glioma cell line, which was not observed with *B. saulcyi*. This difference could be attributed to the high density or presence of blocking channels in these communication networks (12).

In another study, the cytotoxic effect of *O. doriae* venom on 1321N1 cells was confirmed using the MTT assay, which was reduced by ion channel blockers, suggesting that the venom acts primarily via sodium channels. Notably, they observed reduced cytotoxicity at 48 hours, possibly due to venom degradation in aqueous environments (3). Consistently, we observed that venom cytotoxicity peaked at 24 hours and declined at 48 and 72 hours, possibly due to cellular recovery, venom degradation, or cell proliferation. In our study, *O. doriae* venom exhibited a dose- and time-dependent cytotoxic effect on MCF-7 cells. In our study, a significant decrease in the viability of MCF7 cells was also observed in the first 24 hours.

Results from a study on *O. bidentatus* showed that MCF-7, A549 and AGS cells responded significantly to cytotoxicity (1).

Euscorpius mingrelicus was also associated with reduced MCF-7 proliferation (10). One study also reported that the anticancer treatment of *Hottentotta saulcyi* significantly reduced MCF-7 cell viability and apoptosis (≈62%) (22). These conclusions indicate that the chemical composition and toxins from one species to another, as well as the physiological characteristics and genetic lines of different cells, determine the intensity and direction of the effect.

A study found that *Androctonus australis* venom had cytotoxicity against MCF-7 cells with an IC_{50} of 19.71 μg/mL (13). In our study, IC_{50} values were 4.915 μg/mL at 24 hours, 32.72 μg/mL at 48 hours and 3.749 μg/mL at 72 hours. The results of the multiple comparison test (Tukey's post hoc analysis) after one-way analysis of variance were performed to evaluate the differences in MCF-7 cell viability between different concentrations of crude *O. doriae* venom between 24, 48 and 72 hours, which showed significant differences ($p< 0.05$)

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mainly at 24 hours, confirming the dose-dependent cytotoxic effect of the venom during the initial exposure. As a result, a 24 hour dose-response curve was drawn.

In our study, the viability of MCF7 cells at 250 and 500 µg/mL venom is higher than that at 125 µg/mL (at 48h). Considering that the proteins of the arachnid body maintain their properties in the first 24 hours, after 48 and 72 h, their effectiveness weakens and disappears. Therefore, it justifies unexpected results, such as higher viability of the cells at 250 and 500 µg/mL venom than that at 125 µg/mL after 48h.

The higher IC_{50} at 48 hours suggests reduced venom activity or increased cellular resistance over time. This suggests that the cytotoxic effect decreases over time, either because cells can adapt or because the toxin is gradually eliminated or degraded. In 72 hours, it can be said that mortality is not due to the effect of the venom and the IC_{50} is due to the scorpion venom, but rather mortality is due to the long duration of incubation and the reduction in the amount of culture medium.

Such a trend is consistent with some other studies that show a decrease over time, and suggests that the dose or frequency of treatment in a program with programmed toxins should be adjusted to maintain anticancer efficacy. In contrast, some other studies have shown that despite low IC_{50s} for lines such as MCF-7, the magnitude of the response to toxins can be compared with other models, which could be due to differences in sampling, venom preparation methods, or assays (1, 8, 10, 22).

In general, the available evidence from different scorpions suggests that their toxins can target cancer cells, which can be effective against cancer cells, but the intensity of this effect and its persistence depend on the scorpion species, cell line, and experimental conditions.

Conclusions

In this study, we demonstrated that the crude venom of *Odontobuthus doriae* exhibits cytotoxic effects on MCF-7 breast cancer cells, significantly reducing cell viability in a dose- and time-dependent manner. The mortality rate increased after 24 hours of exposure across a concentration range of 0.2 to 500 µg/mL, accompanied by a corresponding decrease in cell survival. Notably, 24 hours after venom exposure, cell proliferation ceased, and the number of viable cells declined. However, as the cytotoxic effect diminished over time, likely due to venom denaturation in aqueous solution at elevated temperatures, surviving cells resumed proliferation. This reduction in cytotoxicity observed at 48 hours highlights the importance of a 24 hour exposure time for evaluating venom-induced cytotoxicity.

Overall, these findings suggest that *O. doriae* crude venom contains bioactive components with potential as anticancer agents. Our results clearly show that *O. doriae* has strong cytotoxic potential in the early time frames, but its effect has been studied at longer time points. Also, the discrepancies reported with other studies suggest that careful investigation of the active compounds, molecular mechanisms, and selection in different models is necessary to be able to approach a possible clinical translation. Future studies will aim to isolate and identify the specific fraction(s) responsible for these cytotoxic effects.

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Ethical considerations

This study has been reviewed and approved by the School of Public Health, Tehran University of Medical Sciences (TUMS) ethics committee, and has been registered with the code IR.TUMS.AEC.1402.006.

Conflict of interest statement

The authors declare there is no conflict of interest.

References

1. Salehi-Najafabadi Z, Goudarzi HR, Sajadi M (2022) Evaluation of in vivo lethality and in vitro cytotoxic effect of *Odontobuthus bidentatus* scorpion venom. Arch Razi Inst. 77(1): 29–36.
2. Moradi M, Solgi R, Vazirianzadeh B, Tanzadehpanah H, Saidijam M (2018) Scorpion venom and its components as new pharmaceutical approach to cancer treatment, a systematic review. Int J Pharm Sci Res. 9(7): 2604–2615.
3. Ghasemi S, Salarian AA, Mirakabadi AZ, Jafarinejad S, Ghazi-Khansari M (2017) Effect of crude venom of *Odonthobuthus doriae* scorpion in cell culture using ion channel modulators. Iran J Pharm Res. 16 (2): 648–652.
4. Dezianian S, Zargan J, Goudarzi HR, Noormohamadi AH, Mousavi M, Alikhani HK, Johari B (2020) In-vitro study of *Hottentotta schach* crude venom anticancer effects on MCF-7 and Vero cell lines. Iran J Pharm Res. 19(1): 192–202.
5. Zargan J, Sajad M, Umar S, Naime M, Ali S, Khan HA (2011) Scorpion (*Odontobuthus doriae*) venom induces apoptosis and inhibits DNA synthesis in human neuroblastoma cells. Mol Cell Biochem. 348 (1–2): 173–181.
6. Akef HM (2019) Anticancer and antimicrobial activities of scorpion venoms and their peptides. Toxin Rev. 38(1): 41–53.
7. Desales-Salazar E, Khusro A, Cipriano-Salazar M, Barbabosa-Pliego A, Rivas-Caceres RR (2020) Scorpion venoms and associated toxins as anticancer agents: update on their application and mechanism of action. J Appl Toxicol. 40(10): 1310–1324.
8. Salama W, El-Naggar SA, Elnewehy A (2023) Biochemical characterization and in vitro cytotoxic activity of the Egyptian scorpion *Leiurus quinquestriatus* whole body extract. Egypt J Cancer Biomed Res. 7(3): 57–63.
9. Wali A, Wubulikasimu A, Mirzaakhmedov S, Gao Y, Omar A, Arken A, Yili A, Aisa HA (2019) Optimization of scorpion protein extraction and characterization of the proteins' functional properties. Molecules. 24(22): 4103.
10. Kayhan NY, Öcal İÇ, Akdeniz M (2022) Investigation the cytotoxic and anti-proliferative effects of crude venom of *Euscorpius mingrelicus* (Scorpiones: Euscorpiidae) scorpion. Eur J Biol. 81(2): 117–124.
11. Keshavarz Alikhani H, Zargan J, Bidmeshkipour A, Zamani E, Mosavi M, Heidari A, Hajinoormohammadi A (2021) Iranian scorpion (*Odontobuthus bidentatus*) crude venom change the redox potential of MCF-7 breast cancer cell line and induce apoptosis. Mod Med Lab J. 4(2): 28–35.
12. Salarian AA, Jalali A, Mirakabadi AZ, Vatanpour H, Shirazi FH (2012) Cytotoxic effects of two Iranian scorpions *Odontobuthus doriae* and *Bothutus saulcyi* on five human cultured cell lines and fractions of toxic venom. Iran J Pharm Res. 11(1): 357–367.
13. Nafie MS, Abdel Daim MM, Ali IA, Nabil ZI, Tantawy MA, Abdel-Rahman MA (2020) Antitumor efficacy of the Egyptian scorpion venom *Androctonus Australis*: in vitro and in vivo study. J Basic Appl Zool. 81 (1): 8.

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14. Zargan J, Sajad M, Umar S, Naime M, Ali S, Khan HA (2011) 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.
15. Zargan J, Umar S, Sajad M, Naime M, Ali S, Khan HA (2011) 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.
16. Kwon NY, Sung HK, Park JK (2025) Systematic review of the antitumor activities and mechanisms of scorpion venom on human breast cancer cells lines (In vitro study). J Clin Med. 14(9): 3181.
17. Mata de los Rios N, Gastelum-Arellanez A, Clement H, Álvarez-Cruz K, Romero-Terrazas D, Alvarado-González C, Hinojos-Gallardo LC, Corzo G, Espino-Solis GP (2025) Ion-channel-targeting scorpion recombinant toxin as novel therapeutic agent for breast cancer. Toxins. 17(4): 166.
18. El-atti MA, El-Badri N, El-Qassas J (2025) In vitro study of anti-cancer properties of Egyptian scorpion (*Leiurus quinquestriatus*) venom on triple negative human breast cancer cell line MDA-MB-231. Bull Fac Sci Zagazig Univ. 4: 108–118.
19. Ghodeif SK, El-Fahla NA, Abdel-Rahman MA, El-Shenawy NS (2025) Arthropod venom peptides: Pioneering nanotechnology in cancer treatment and drug delivery. Cancer Pathog Ther. 4: 81–97.
20. Lafnoune A, Chbel A, Darkaoui B, Wahnou H, Nait Irahal I (2025) Invertebrate venoms: A treasure trove of bioactive compounds with anticancer potential. Arch Toxicol. 99(7): 2685–2698.
21. El-Qassas J, Abd El-Atti M, El-Badri N (2024) Harnessing the potency of scorpion venom-derived proteins: applications in cancer therapy. Bioresour Bioprocess. 11 (1): 93.
22. Nosouhian M, Rastegari AA, Shahanipour K, Ahadi AM, Sajjadieh MS (2024) Anticancer potentiality of *Hottentotta saulcyi* scorpion curd venom against breast cancer: an in vitro and in vivo study. Sci Rep. 14(1): 24607.
23. Rajput SS, Mohan H, Jadhav E, Sonone SS, Nagar V, Singh A, Chopade RL, Awasthi KK, Sankhla MS (2021) Therapeutic potential of scorpion venom in cancer treatment as anticancer agent: A review. J Forensic Med Toxicol. 38(2): 105–117.
24. Barahoei H, Navidpour Sh, Aliabadian M, Siahsarvie R, Mirshams O (2020) Scorpions of Iran (Arachnida: Scorpiones): Annotated checklist, DELTA database and identification key. J Insect Biodivers Syst. 6 (4): 375–474.